Peat alternatives in the sand-based rootzone mixture for golf turfgrass growth

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À minha família e
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Resumo

A turfa é um substrato muito importante no setor agrícola, ornamental, em campos de golfe, entre outros, todavia a sua extração tem um elevado impacto ambiental. Por este motivo, é necessário encontrar materiais alternativos que sejam mais económicos, produzidos localmente e mais sustentáveis. Os objetivos deste trabalho consistiram em estudar três corretivos orgânicos, alternativos à turfa, na instalação de relvados de golfe; avaliar o efeito do seu azoto residual no crescimento das plantas e identificar as possíveis infestantes. Os corretivos orgânicos testados foram a turfa (PT, controlo), lamas de ETAR co-compostadas com casca de pinheiro (NA), composto orgânico verde (NV) e "terras" de cortiça (CE). As gramíneas estudadas foram *Agrostis stolonifera* L. e *Lolium perenne* L.. Na avaliação da biomassa aérea produzida efectuaram-se cortes semanais e determinaram-se os respectivos pesos secos. Posteriormente, avaliaram-se os macro e micronutrientes presentes na biomassa. Nos solos, analisou-se o teor de matéria orgânica, pH, condutividade eléctrica e a acidez de troca, bem como os macro e os micronutrientes extraíveis e as bases de troca. Os resultados demonstraram que as gramíneas que cresceram na mistura NA tiveram uma produção de biomassa aérea maior que na mistura PT (controlo), seguida da mistura NV. Estes dois corretivos orgânicos foram os que disponibilizaram mais azoto na ausência de fertilização. Verificaram-se possíveis efeitos de antagonismo entre elementos, designadamente no cálcio e magnésio, devido ao excesso de nutrientes nas misturas, como o potássio. As infestantes observadas eram eudicotiledóneas e emergiram do corretivo orgânico CE. O seu controlo pelo corte seria eficaz, com a excepção do género *Sonchus* spp.. A mistura onde as plantas tiveram um menor afilhamento foi a mistura CE e as misturas NA e NV induziram um bom afilhamento, tal como a mistura PT.

Palavras-chave: *Agrostis stolonifera* cv. 'Penn-A4'; *Lolium perenne* cv. ´Benchmark'; infestantes; turfa; corretivos orgânicos.

Abstract

Peat is a very important growing media in the agricultural and ornamental sectors, on golf courses, among others, but its extraction has a high environmental impact. For this reason, it is necessary to find more economical, locally produced and more sustainable alternative materials.The objectives of this work were to study three organic amendments alternatives to peat; to evaluate the effect of their residual nitrogen in the plant growth and to identify possible existing weeds. The organic amendments tested were peat (PT, control), sewage sludge compost with pine bark (NA), organic green compost (NV) and cork "earth" (CE). The grasses studied were *Lolium perenne* L. and *Agrostis stolonifera* L.. To obtain the aerial biomass production, shoot harvesting was made weekly and the dry matter was weighted. Posterior macro- and micronutrient present in the biomass was evaluated. Regarding the rootzone mixtures, the organic matter content, pH, electrical conductivity and the exchangeable acidity were analyzed as well as the extractable macro and micronutrients, and the exchangeable bases. The results showed that the grasses that grew in the rootzone mixtures with NA had the higher aerial biomass production, comparing with peat, followed by the rootzone mixture with NV. These two organic amendments were those who had higher amounts of nitrogen (N) available in the absence of N fertilization. Possible effects of antagonism between elements, namely calcium and magnesium, were due to the excess of nutrients in the rootzone mixtures, such as potassium. The weeds observed were eudicotyledonous and emerged from the organic amendment CE. Its cut would be effective, in order to control them, with the exception of the genus *Sonchus* spp.. The rootzone mixture where the plants had lower development and tillering was the CE and the rootzone mixtures NA and NV induced good development, as well as the rootzone mixture PT.

Keywords: *Agrostis stolonifera* cv. 'Penn-A4'; *Lolium perenne* cv. 'Benchmark'; weeds; peat; organic amendments.

Resumo alargado

O golfe é um desporto que tem um papel importante na sociedade e no ambiente, uma vez que está diretamente associado à natureza e contribui para a melhoria da saúde humana (Farrally *et al.,* 2003). Os campos de golfe ocupam vastas áreas. À sua construção e manutenção estão associados um elevado impacto ambiental, visto que é necessário destruir ecossistemas para que estes sejam instalados e são utilizadas elevadas quantidades de água, pesticidas e fertilizantes durante longos períodos de tempo. Devido a estes impactos negativos várias organizações em diferentes países estão a adotar metodologias de modo a promover a sustentabilidade deste desporto (Guzmán & Fernández, 2014). Contudo, após a sua instalação, estes podem contribuir para a conservação da vida animal e para a melhoria da qualidade do ar, uma vez que, um campo de golfe consegue renovar 13 milhões de toneladas de ar por ano (USGA, 2015; Sewell, 2019).

O golfe tem um elevado impacto nas economias locais. Este desporto está diretamente associado ao turismo que é um dos maiores sectores em crescimento em Portugal, pois a emergência de novos campos de golfe está associada ao desenvolvimento de novos *resorts* que, por sua vez, aumentam o desenvolvimento económico (Farrally *et al.,* 2003; Andrade & Atão, 2015).

Das várias áreas que compõem um campo de golfe, o *green* é a área mais importante. Vários aspectos têm que ser tidos em conta: a sua localização, visibilidade, tamanho, contudo o mais importante é a construção (Watson, 2012). A *United States Golf Association* (USGA) é uma organização que formulou métodos recomendados para a construção de *putting greens,* assim sendo, todos os aspectos físicos dos componentes usados na sua construção, devem obedecer aos valores recomendados.

A instalação de relva pode ser feita por dois métodos, tapetes pré-cultivados ou sementeira direta. Em ambos os casos, na preparação da camada em que o *green* vai ser instalado, os corretivos mais usados para misturar com a areia são a turfa, composto ou materiais inorgânicos de composição mineral variada (McCoy, 2013). A areia é usada pois fornece uma superfície regular, evita a compactação e tem uma elevada capacidade de drenagem (Vaughn *et al.*, 2018). Apesar da turfa ser o corretivo orgânico mais usado devido, às suas propriedades físicas, nomeadamente a sua elevada capacidade de retenção de água, é um recurso natural não renovável (Taylor & Blake, 1979; McCoy, 2013).

As turfeiras são ecossistemas únicos e insubstituíveis que requerem conservação (Barkham, 1993). Devido às suas condições específicas, não se encontram em todos os países dado que são necessárias: condições de anaerobiose, baixas temperaturas e a presença de várias espécies vegetais, das quais se destaca o musgo do género *Sphagnum* spp. (Waddington & Price, 2000).

A extração de turfa é uma atividade com elevado impacto ambiental, pelo que diversos países têm vindo a adotar políticas no sentido de reduzir o seu uso no setor agrícola e ornamental, nomeadamente o Reino Unido (Spain, 2018). Com a crescente preocupação da sociedade para com o ambiente e as alterações climáticas, a procura de alternativas ao uso de turfa está focada para materiais que sejam produzidos localmente, uma vez que estão mais disponíveis e são economicamente mais acessíveis (Ortega *et al*., 1996).

É neste contexto que surge o presente trabalho, onde se pretende encontrar corretivos orgânicos, de origem nacional, que demonstrem características adequadas para substituir a turfa, na formulação das misturas utilizadas na instalação de relvados. Assim, testaram-se os seguintes corretivos orgânicos: turfa (PT, controlo); lamas de ETAR co-compostadas com casca de pinheiro (NA), composto orgânico verde (NV) e "terras de cortiça" (CE).

As gramíneas utilizadas foram as espécies *Agrostis stolonifera* L. e *Lolium perenne* L., que se propagam por semente, o que torna a sementeira fácil e quando instaladas, a propagação é vegetativa, por estolhos. A espécie *A. stolonifera*, é a gramínea mais usada nos *greens* dos campos de golfe, uma vez que tem uma elevada tolerância a cortes muito baixos, um crescimento rápido e compõe tapetes densos (Nordick, 2009; Tee2Green, 2011). O táxone *L. perenne* é uma das gramíneas mais usadas no mundo, em específico nos campos de golfe, o seu uso está a aumentar porque também tolera cortes baixos, tem uma densidade moderada e a textura das folhas é média (Oregon Ryegrass, 2013; McCarty, 2000).

Das várias características ideais que um substrato deve ter (físicas, físico-químicas e químicas), a ausência de sementes de infestantes é uma das mais importantes (Cunha *et al.*, 2010). Como o corretivo orgânico "terras de cortiça" é composto por uma parte significativa de solo, oriundo do seu local de origem, é provável que contenha um banco de sementes, uma vez que se trata de um resíduo da indústria da cortiça que não foi tratado, ou seja, não foi desinfetado. A presença de infestantes nos campos de golfe é um problema pois estas competem por nutrientes, luz e água, podendo dizimar todo o relvado instalado. Provocam ainda, a perda de uniformidade do relvado e como consequência a sua qualidade ornamental, que é uma das finalidades (Maciel *et al.*, 2008).

Assim, os objetivos deste trabalho foram: i) comparar o efeito de três corretivos orgânicos disponíveis em Portugal com o efeito da turfa, no crescimento de *Lolium perenne* cv. 'Benchmark' e *Agrostis stolonifera* cv. 'Penn-A4'; ii) observar o efeito do azoto residual, dos corretivos orgânicos, no crescimento das gramíneas e iii) pesquisar, identificar e caracterizar as infestantes presentes.

O presente estudo foi realizado no Horto de Química Agrícola "Professor Boaventura de Azevedo", localizado no Instituto Superior de Agronomia, Universidade de Lisboa, na Tapada da Ajuda.

Para a avaliação da biomassa área produzida, efectuaram-se cortes semanais e determinaram-se os respectivos pesos secos. Posteriormente, avaliaram-se os macro e micronutrientes presentes na mesma. Relativamente aos solos, analisou-se o teor de matéria orgânica, pH, condutividade eléctrica e a acidez de troca, bem como os macro e os micronutrientes extraíveis, e as bases de troca.

Os resultados demonstraram que as gramíneas que cresceram na mistura contendo o corretivo NA tiveram uma produção de biomassa aérea maior do que na mistura com turfa (PT), seguida da mistura NV. As plantas tiveram um menor afilhamento na mistura CE, enquanto que as misturas NA e NV induziram um bom afilhamento, tal como a mistura controlo (PT).

A adição dos corretivos orgânicos NA e NV aumentaram significativamente o teor de nutrientes na mistura à base de areia, usada na instalação dos relvados. Foi possível verificar o efeito do azoto residual destes corretivos orgânicos, no crescimento das espécies estudadas, uma vez que foram os que disponibilizaram mais azoto na ausência de fertilização. Verificaram-se possíveis efeitos de antagonismo em elementos, designadamente no cálcio e magnésio, devido ao excesso de nutrientes nas misturas como o potássio.

Considerando o custo - benefício da utilização dos vários corretivos orgânicos estudados, o corretivo orgânico NA (lamas de ETAR co-compostadas com casca de pinheiro) demonstrou ser o melhor, seguido do corretivo orgânico NV (composto verde).

A maioria das infestantes presentes emergiu do corretivo orgânico CE, sendo todas dicotiledóneas. Devido à frequência e à baixa altura dos cortes que são efetuados nos campos de golfe, não seria necessário recorrer ao uso de herbicidas para gerir o seu desenvolvimento, uma vez que são espécies terófitas (espécies anuais) e a sua regeneração seria afetada. Contudo, o género *Sonchus* spp. representa uma excepção, uma vez que se trata de uma proto-hemicriptófita e, após os cortes, esta voltaria a regenerar.

Duas espécies de fungos foram identificadas no decorrer dos ensaios, um deles o causador da doença "*Leaf Spot*" e o outro um fungo saprófita associado ao corretivo orgânico CE.

Com os resultados obtidos, são necessários mais estudos no campo, para avaliar a produção de biomassa, o desenvolvimento e o afilhamento das espécies das gramíneas usadas, assim como, avaliar as alterações físicas, químicas e biológicas que poderão ocorrer, na camada onde o relvado está instalado.

Com os teores de nutrientes naturalmente presentes nos corretivos orgânicos, seria interessante analisar o efeito de diferentes quantidades de fertilização ou de diferentes misturas entre os corretivos orgânicos, na produção de biomassa.

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Abbreviations and acronyms list

e. g. -	for example
i. e. -	in other words
t -	tonne
m -	meter
mm -	milimeter
g -	grams
L -	liter
mL -	milliliter
kg -	kilogram
km -	kilometer
C -	celsious
ha -	hectare
SS -	silica sand
PT -	peat
NA -	Naturanat® (sewage sludge and pine bark)
NV -	Nutriverde Premium® (green compost)
CE -	cork "earth"
MWHC -	Maximum Water Holding Capacity
CEC -	cation exchange capacity
WWTP -	Waste-Water Treatment Plant
OM -	organic matter
EC -	electrical conductivity
CO_2 -	carbon dioxide
N -	nitrogen
P_2O_5 -	phosphorus
K_2O -	potassium
Ca -	calcium
Mg -	magnesium
S -	sulfur
Na -	sodium
Fe -	iron
Mn -	manganese
B -	boron
Zn -	zinc
Cu -	copper
Mo -	molybdenum
Pb -	lead
Cr -	chromium
Ni -	nickel
Cd -	cadmium

1. Introduction

Peat is a non-renewal natural resource that, due to its physical properties, is the most used material in gardens, golf courses, sowing and installation of grass mats (McCoy, 2013).

Peat bogs are unique and irreplaceable ecosystems that require conservation (Barkham, 1993). Since peat extraction is an activity with high environmental impact, some countries are taking measures in order to reduce the use of this organic material in the agricultural and ornamental sectors, as in the United Kingdom (Spain, 2018). This country is one of the several importers of peat provided from the Republic of Ireland, which aims to eliminate its use in the gardening industry until 2030 (Simões, 2017; Spain, 2018).

With the augment of the social awareness concerning the environment and the climatic changes that are being felt all around the world, the search for finding alternatives to the use of peat is focused to locally produced materials, since that these are more available and economically more accessible (Ortega *et al.*, 1996).

The perennial ryegrass (*Lolium perenne* L.) is the most used grass species in the world and its use on golf courses is increasing due to its characteristics: fairly high shoot density, medium leaf texture, bright green color and tolerance of low mowing heights (Oregon Ryegrass, 2013; McCarty, 2000). The creeping bentgrass (*Agrostis stolonifera* L.) is the grass primarily used for golf courses putting greens, since it has a high tolerance to low mowing heights, has an aggressive growth habit, creates dense grass mats by spreading its stolons, produces narrow leaves originating a very fine-texture mat and the leaves are dark green colored (Nordick, 2009; Tee2Green, 2011).

The organic materials that were selected for this work were: Naturanat®, a residue that is produced in the Waste Water Treatment Plant of Maia Municipality, Portugal, and it results from the co-composting of sewage sludge and pine bark; Nutriverde Premium®, an organic product that is produced in the Composting Unit Green Residues of São Brás de Alportel, Portugal, and it results from the composting of green residues provided by parks, gardens and golf courses; and cork "earth" that is one of the main residues of the cork industry and it is composed by granulated low-quality cork containing a substantial amount of soil. At the present time, there is no destiny to this last product. By reusing these organic wastes, the circular economy could be promoted by increasing the valorization of these materials and also the environmental sustainability of these industries.

It is in this context that it is intended to find at least one organic material, of national origin, which has the characteristics that will allow it to be a good candidate to replace peat that is used as an organic amendment in the rootzone sand-base mixture, for putting greens.

The aims of the study were: i) to compare the effect of three organic amendments compared to peat, on *Lolium perenne* cv. ‘Benchmark’ (perennial ryegrass) and *Agrostis stolonifera* cv. ‘Penn-A4’ (creeping bentgrass) growth; ii) observe the organic amendments nitrogen residual effect in the plants growth and iii) surveyed, identification and characterization of weeds.

2. State of Art

2.1. Golf in the world and in Portugal

2.1.1. The importance of golf

Golf is a sport that not only increases human health due to its aerobic component that can reduce stress, but also has an important social and environmental contribution (Farrally *et al.,* 2003). Unlikely other sports, this one covers high areas of land, an estimated 147.553.000 km^2 of land occupied, in 209 countries, around the world (Klein, 2019).

There is a high environmental impact associated with the golf courses construction and maintenence since it is necessary to destroy ecosystems so that they can be installed and high amounts of water, pesticides and fertilizers are used over long periods of time. Due to these negative impacts, several organizations in different countries are adopting methodologies to promote the sustainability of this sport (Guzmán & Fernández, 2014). However, after installation, they can contribute to the conservation of animal life and the improvement of air quality, since a golf course can renew 13 million tons of air per year (USGA, 2015; Sewell, 2019).

At the end of 2018, there were 38.864 golf courses in the world, 8.940 in Europe (7% of total land on earth) and 106 in Portugal. Europe is the busiest continent in terms of golf development, where since 2014 until 2018, 99 new golf courses opened representing 24% of the world golf development during that time (Klein, 2019).

Golf has a big impact on local economies. This sport is directly associated with tourism, which is one of the higher growing sectors in Portugal, as the emerging of new golf courses is associated with the development of resorts which increase the economic development (Farrally *et al.,* 2003; Andrade & Atão, 2015).

In 2015 the worldwide demand for golf was made up of 1 million travels and it was estimated and an increase of 7% per year. The leisure-motivated travels (including golf) to Portugal, being the current resorts and futures ones the primarily target of tourists and golfers, will have a central role in affirming this country as a golf destination (Catarino, 2010). In Portugal, the golf tourism generates around 2 thousand million Euros per year that represents 1.25% of the National Gross Domestic Product (GDP) (14% of the touristic DGP) (Andrade & Atão, 2015).

Golf is not only associated with tourism but also with nature. One of the reasons that golf is much appreciated and sought is because of the need for humans to be connected with nature (PortugalGolf, 2005). This rapid golf growth as required the analysis made by several physical and biological sciences, to determine the role of golf in the society and its impacts in the environment (Farrally *et al.*, 2003).

Since golf courses cover a significant area of green space and have prohibit areas for playing, they provide shelter and contribute for the conservation of wildlife, includes natural trees and vegetation, the turf protects the top soil from water and wind erosion; improves air quality since a golf course can filtrate 13 million t of air per year and the golf courses improves community aesthetics (USGA, 2015; Sewell, 2019).

2.1.2. The use of organic amendments in the rootzone sand mixtures

The golf course is a complex and dynamic set of green areas that involves knowledge in many sciences such as engineering, horticulture, agronomy, wildlife management, hydrology, land planning, environmental and soil science, in order to provide a high-quality playing surface with less quantity of inputs of pesticides, water and fertilizers (Farrally *et al*., 2003).

The golf course is composed by several aeras being the “green” the most importat one (Figure 1).

Figure 1. The different areas that constitute a golf course (adapted from Sewell, 2019).

Many aspects have to be taken into consideration regarding the greens such as its placement, visibility, size, shape but most importantly its construction (Watson, 2012). The United States Golf Association (USGA) is an organization that founded a standard method of construction for putting greens and it had been used as a guideline all around the world, for nearly 60 years. The green is composed mainly of four layers: the putting green cavity (where the putting green is going to be installed), the drainage system (where the drainage pipes are installed), the intermediate layer (where gravel is placed to cover the drainage system) and the rootzone layer (sand-based mixture where the turf is laid or the seed sown) (USGA, 2018; Watson, 2012) (Figure 2).

Figure 2. The four layers that compose a putting green: the putting green cavity (1), the drainage system (2), the intermediate layer (3) and the rootzone layer (4) (adapted USGA, 2018).

The top layer (4) is the most important one and it has several recommendations for each aspect of its components. The height of the layer should be 300 millimeter in depth, the sand particles, used in the sand-based mixture, should have any diameter between 0.05 - 2 millimeters and should be chemically inert such as silica minerals including quartz (USGA, 2018). The sand is used to provide a uniform and smooth surface, prevent compaction and to allow rapid water drainage (Vaughn *et al.*, 2018).

However, since sand has poor water holding capacity and low nutrient retention capacity, it needs to be amended. Therefore, it is usually mixed with an organic or an inorganic material (Vaughn *et al.*, 2018). An organic amendment is an organic material from animal or plant origin, intended to increase the content of organic matter in the soil and plays an important role in soil fertility as well as in its physical, chemical and biological aspects (DRAPC, 2002).

Studies have been carried out to determine the best ratio of sand: organic material and addictions of 5% up to 20% by volume show that there is an increase in the water and nutrient retention. By increasing the water retention, the risk of drought is reduced and the period between irrigation can be extended and, by improving the nutrient retention, the risk of nutrient-induced stress decreases between fertilizations (McCoy, 2013).

Also both organic and inorganic materials have recommendations according to their origin, their particle size, physical and physicochemical characteristics (USGA, 2018). Peat is the most used organic material in the rootzone mixture of putting greens and currently there are studies being made in order to substitute peat with other organic materials, such as by-products of farms and cities like composts and municipal biosolids that are more carbon-neutral than peat (Vaughn *et al.,* 2018; Farrally *et al*., 2003).

2.1.3. The influence of weeds in the turfgrass quality

With the increase of the concern for the environment protection in golf courses, regarding water management, conservation of wildlife and the use of pesticides, the need to have better management practices is necessary. Pesticides are heavily used to control pests and weeds, in order to maintain the golf course playable and healthy for the golfers (Sewell, 2019). There are six aspects that classify the quality of the turf grass: growth habit, uniformity, density, smoothness, texture and color (Arteca, 2015). The regular mowing of the plants increases their tillering which turns to greater shoot density, resulting in a denser and uniform turf mat (Beard, 1973).

A weed is a plant whose presence is not wanted, nevertheless it is to take into consideration that the same plant can be considered a weed in one golf course but not in another. Although they are an important part of the ecosystem, their presence is a serious problem since they are very hard to control (indeed most grass weeds belong to the same clade as the grass species used in golf turfs – monocotyledoneae) (Waters, 2019).

Usually weeds life cycle mimic the crop where they are inserted and regarding golf courses, the weeds that develop are regularly perennial. There are many reasons why weeds are not tolerated, being economical and aesthetical the main two reasons in golf courses (Merfield, 2019).

Weeds compete for nutrients, light and water and their presence causes a loss of uniformity, density and color, resulting in the reduction of ornamental quality and originate playability issues (negative aesthetic and economic impacts, respectively) (Maciel *et al.*, 2008). If the weather does not favor the normal growth of an installed grass mat, regardless the area of the golf course, or in a new sowed area, the weeds can occupy any gap between the turfs and thrive (Waters, 2019).

Golf courses have naturally present a seed bank in the soil from their own grass species and it is easier to control weeds by preventing their arrival and emergency, than to control them after germination and being installed (Merfield, 2019).

Thus it is necessary to endeavor that no new weed seeds or weed propagules are introduced in the golf course. This can be achieved by guaranteeing that the grass seeds are not contaminated with other seeds, ensure that the organic amendments used in the sand-based mixtures were sterilized and weed seed/propagules free and make sure that the grass mat that is going to be installed, was grown in a soil that did not have itself a seed bank and weed seedlings.

2.2. The grass species used

The grass species used in this study were *Lolium perenne* L. and *Agrostis stolonifera* L. due to their botanical characteristics.

The perennial ryegrass (*L. perenne*) is the most used grass species in the world and its use on golf courses is increasing since it can tolerate low mowing heights, has a fairly high shoot density, medium leaf texture and the leaves are bright green in color (Oregon Ryegrass, 2013; McCarty, 2000). The creeping bentgrass (*A. stolonifera*) is the grass primarily used for golf courses putting greens, given that it has a high tolerance to low mowing heights, has an aggressive growth habit, creates dense grass mats by spreading its stolons, produces narrow leaves originating a very fine-texture grass mat and the leaves are dark green in color (Nordick, 2009; Tee2Green, 2011).

2.2.1. *Lolium perenne* L. biology and ecology

Taxonomic Tree (CABI, 2018):

Kingdom: Plantae
- Division: Spermatophyta
  - Subdivision: Magnoliophyta
    - Clade: Monocoltyledoneae
      - Order: Cyperales
        - Family: Poaceae
          - Genus: *Lolium* L.
            - Species: *Lolium perenne* L.

Scientific name: *Lolium perenne* cv. ‘Benchmark’

Common name: Perennial ryegrass

This plant is native from Europe (Figure 3), Asia and North Africa (Oregon Ryegrass Growers Comission, 2013). It is native of Portugal and classified as exotic in the Madeira and Azores archipelago (Flora.On, 2012).

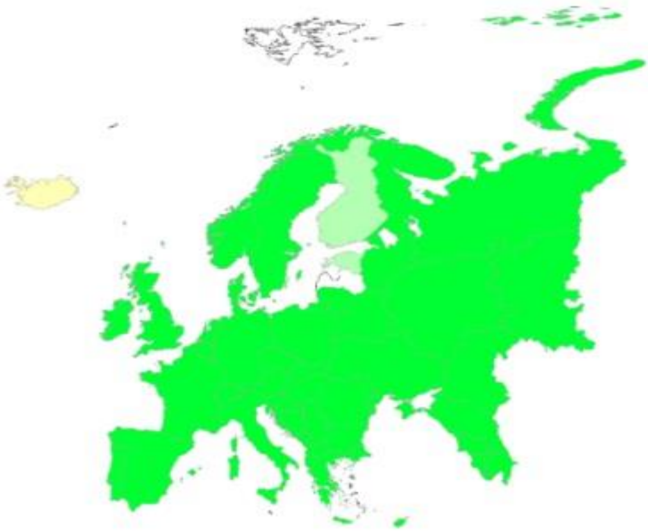


Figure 3. *Lolium perenne* European distribution. The green color indicates native occurrence, the light green indicates naturalized occurrence and the yellow indicates that the plant was cultivated (adapted from iFlora, 2019).

Lolium perenne is a cool-season grass – C3, and despite being well adapted to temperate and transition climate, this species is well adapted to regions with arid and hot climate (Landmark, 2011). The best range of temperature of its optimum growth is between 18 - 20°C (CABI, 2018).

This species adapts well to several types of soil but it prefers soils with a medium texture and tolerates acidic and alkaline soils, with a pH range of 5.2 and 8.0. It tolerates compacted soil and temporarily flooded areas but does not tolerate salinity (CABI, 2018).

It is a perennial herbaceous plant from the Poaceae family (clade monocoltyledoneae). It is a self-supporting terrestrial plant, hemicryptophyte according to the Raunkiaer life form ranking, *i. e.* their shoot buds are located on the ground surface (Flora.On, 2012; Sullivan, 1992). It has a vertical growth, reaching between 15 to 80 cm (up to 100 cm), emits erect and cylindrical culms, short rhizomes and it develops by tillering (Tela Botanica, 2019; Monteiro *et al.*, 2014).

The leaves are narrow, bright green, flat or bent. Each is composed of a blade, a free margin sheath that are reddish when young due to the presence of anthocyanins, a membranous ligule truncate, with 0,5-1,5 mm in length and a pair of short, non-evident pair of auricles (Oregon Ryegrass Growers Comission, 2013; Monteiro *et al*., 2014).

The flowers are hermaphrodite and the pollination is made through the wind (anemophily pollination) (Tela Botanica, 2019).The flowering occurs from May to August and the fruit that is produced is of the caryopses type (Figure 4) that has no after-ripening phase. This means that the process of germination occurs immediately after sowing - germinates 5 to 10 days after sowing (CABI, 2018; Oregon Ryegrass, 2013).

Figure 4. The fruit produced by perennial ryegrass (Leica®, model S9i). Image processed by the software LAS V. 4.12.

2.2.2. *Agrostis stolonifera* L. biology and ecology

Taxonomic Tree (CABI, 2018):

Kingdom: Plantae

Division: Spermatophyta

Subdivision: Magnoliophyta

Clade: Monocotyledoneae

Order: Cyperales

Family: Poaceae

Genus: *Agrostis* L.

Species: *Agrostis stolonifera* L.

Scientific name: *Agrostis stolonifera* cv. 'Penn-A4'

Common name: Creeping bentgrass

This grass can be found in most of Europe (Figure 5), North Africa, in the Caucasus and in part on West of Asia (UTAD, 2019). It is native of Portugal and the Madeira archipelago and classified as exotic in the Azores archipelago (Flora.On, 2012).

Figure 5. *Agrostis stolonifera* European distribution. The green color indicates the native occurrence and the light green indicates the naturalized occurrence (adapted from iFlora, 2019).

Agrostis stolonifera is a cool-season grass – C3, that has become naturalized in temperate to cold-temperate regions but this species also adapts well to transition, arid and hot climate (A. Pereira Jordão, 2011; Macbryde, 2006). It is well adapted to a variety of habitats and can be found in forests, grasslands, prairies, meadows and lake margins (Esser, 1994; Franco & Afonso, 1998).

It prefers sandy soils, semi wet, tolerates poorly drained conditions and temporarily floods and submergence (Esser, 1994; Monteiro *et al*., 2014).The best range of temperature of its optimum growth is between 15 - 25°C and there are ecotypes that possess some salinity tolerance (Almeida, 2004; Ahmad & Wainwright, 1977).

It is a stoloniferous, fast-growing, perennial, herbaceous plant, from the family Poaceae (clade monocotyledoneae). It is an autotrophic, self-supporting terrestrial plant, proto-hemicryptophyte and geophyte according to the Raunkiaer life form ranking, *i. e.* their shooting buds are located on the ground surface (Flora-on, 2019; Esser, 1994). It has a vertical growth reaching between 0 - 100 cm (up to 150 cm). The stems (culms) are erect and ascending but occasionally decumbent. The vegetative spread is made by emitting stolons (horizontal above ground stems) that produce roots in their knots (Macbryde, 2006).

The leaves are very fine, flat and dark green. Each is composed of a sheet, a blade and an obtuse ligule, truncate to acute, with 2-10 mm in length (Monteiro *et al*., 2014). It does not possess any auricles.

The flowers are hermaphrodites and wind-pollinated (anemophily pollination). The flowering occurs from May to September and the fruit that is produced is of the caryopses type. The seeds are very small (Figure 6), which allows them to be easily dispersed by the wind, water and animals (Esser, 1994). They have no after-ripening phase, which allows them to germinate right after their dispersal - germinates approximately 5 days after sowing, under ideal conditions (Macbryde, 2006).

Figure 6. The fruit produzed by creeping bentgrass: 1) caryopses; 2) caryopses with the glumellas (Leica®, model S9i). Image processed by the software LAS V. 4.12.

2.3. Peat

Peat is an organic decomposed material, composed mostly by *Sphagnum* mosses, herbs and shrubs, produced in a very specific ecosystem; the peat bogs. It is the most used material in gardening, horticulture, ornamental industry and for the production of heat and electricity by burning (Spain, 2018).

Peat has many definitions depending on the country and on the authorities but according to the U.S. Department of Agriculture Soil Classification, peat is “an organic soil that contains a minimum of 20% organic matter” (IPS, 2019).

Due to the conditions where peat is produced such as low oxygen and low temperature, when the plants die they accumulate and decompose in a slow rate. It is because of this fact that the characteristics of peat do not change significantly during the storage period. Its physical characteristics like the water holding capacity and high porosity, and chemical characteristics such as high cation exchange capacity (CEC), pH, low nutrient status, makes peat the organic material with the appropriate conditions to be used as an organic amendment in golf courses and gardens (Kitir *et al*., 2018).

A fresh sample of peat is mainly composed by 80-90% water and the remaining 2-10% composed by solid organic and inorganic material. Peat is classified according to its degree of humification, *i.e.* decomposition, resulting from microbial degradation. This ranking is designated and described in the “von Post Humification Scale” (Table 1) (FAO, 1988).

Table 1. The von Post scale of humification (adapted from Ekono, 1981).

Symbol	Description
H1	Completely undecomposed peat that, when squeezed, releases almost clear water. The plants remain easily identifiable. No amorphous material present.
H2	Almost entirely undecomposed peat that, when squeezed, releases clear or yellowish water. The plants remain still easily identifiable. No amorphous material present.
H3	Very slightly decomposed peat that, when squeezed, releases muddy brown water, but from which no peat passes between the fingers. The plants remain still identifiable and no amorphous material present.
H4	Slightly decomposed peat that, when squeezed, releases very muddy dark water. No peat is passed between the fingers but the plant remains are slightly pasty and have lost some of their identifiable features.
H5	Moderately decomposed peat that, when squeezed, releases very "muddy" water with a very small amount of amorphous granular peat escaping between the fingers. The structure of the plants is quite indistinct although it is still possible to recognize certain features. The residue is very pasty.
H6	Moderately highly decomposed peat with a very indistinct plant structure. When squeezed, about one-third of the peat escapes between the fingers. The residue is very pasty but shows the plant structure more distinctly than before squeezing.
H7	Highly decomposed peat. Contains a lot of amorphous material with very faintly recognizable plant structure. When squeezed, about one-half of the peat escapes between the fingers. The water, if any is released, is very dark and almost pasty.
H8	Very highly decomposed peat with a large quantity of amorphous material and very indistinct plant structure. When squeezed, about two-thirds of the peat escapes between the fingers. A small quantity of pasty water may be released. The plant material remaining in the hand consists of residues such as roots and fibers that resist decomposition.
H9	Practically fully decomposed peat in which there is hardly any recognizable plant structure. When squeezed it is a fairly uniform paste.
H10	Completely decomposed peat with no discernible plant structure. When squeezed, all the wet peat escapes between the fingers.

2.3.1 The importance of peat bogs ecosystems

Peat bogs are complex ecosystems that have an important role in the methane retention and carbon dioxide accumulation. They are one of the major terrestrial carbon accumulation systems in the world (Pereira, 2014; Crill *et al.*, 1988). When the peat is harvest, the gases that were accumulated on the ground are released to the atmosphere, being a serious problem since that this gases contribute to the current climate changes that we face nowadays (Waddington & Price, 2000). These systems not only have the ability to retain methane and to capture carbon dioxide, as they supply and regulate the water cycles, they contribute to the soil formation and regulate its nutrients. They are a genetic resource and a biodiversity refuge (ICNF, 2017).

Studies made about the peat extraction show that, since the peat bogs are non-renewal resources, after the industrial activity, they are abandoned. It is necessary to proceed to the ecological restoration of these wetlands so that they can be, once again, a functional carbon retention system (Waddington & Price, 2000), which is a very sensitive process that can take many years to be achieved (Dale Vitt, 2012).

In Portugal, peat bogs occupy a very small area, being mostly present in the mountain regions, in the Azores archipelago and, punctually, represented in the sub-littoral areas (Mendes & Dias, 2009). The industrial and commercialization of peat is not available so, to use peat in nurseries, Portugal has to import peat from other countries.

2.3.2. Commercialization of peat

The current use of peat is unsustainable. Due to concerns about the environment, countries that explore these ecosystems or that import peat from other countries, are looking for peat-free or reduced products as an alternative. The United Kingdom is one of the countries that wants to reduce the use of peat in the gardening sector and it is aiming for the elimination of peat until 2030 (Spain, 2018).

The three main exporters of peat are Canada (28%), Germany (16%) and Latvia (14%). The three main importers are United States of America (28%), Holand (10%) and France (6.6%). The United Kingdom, in 2017, imported around 4.3% of the world peat imports and it is mainly from Ireland (68%). Portugal, in 2017, contributed around 0.73% of the world peat imports and approximantly 51% is from other countries themselves also peat importers (Spain and Holand). Only 24% of the peat imported is provided by a country that explores peat ecosystems naturaly present, Germany (Simões, 2017).

Peat can be more expensive than other substitutes such as composts, especially if there is a local source of compost that can sell the product in bulk (Government of Ireland, 2019). The reduction of peat use leads to limited supply of peat and consequently, an increase of shipping and purchase costs (Ribeiro *et al*., 2009). Therefore, along with the policy adopted by the United Kingdom, new and more sustainable organic materials are needed to be used in the sand-based rootzone mixture as an organic amendment.

2.4. Composting products

Compost is an organic material that results from the composting process. It consists in the biological degradation of heterogeneous organic material, in order to obtain a stable material with humic substances, free of pathogens, weed seeds and propagules, making it suitable to be used as an organic amendment in the soil (Sempiterno, 2016).

Composting has a positive impact on the environment as it reduces the amounts of methane released to the atmosphere due to the fact that this process reduces de volume of the materials that are exposed to the air, minimizes odors and increases the valorization of wastes and consequently, decreases the waste quantity that are deposited in landfills (Khater, 2015; Sempiterno, 2016).

Incorporating compost in the soils has many benefits: improves the soil structure, ads nutrients to the soil and releases them slowly, stimulates the microbial activity, increase the amounts of organic matter, its water holding capacity and enhances plant growth (Epelde *et al.*, 2018).

There are a number of materials that can go through the process of composting (Table 2) such as farm residues to urban biodegrabable materials (paper and food that can be subject to aerobic and anaerobic decomposition) and urban wastes (waste from households or similar) (Decreto Lei nº 113/2015 of June 15th of Ministério da Economia, 2015; Khater, 2015).

Table 2. Examples of several materials that can use in the composting process (adapted from Sempiterno, 2016).

Materials rich in nitrogen (N)	**Materials rich in Carbon** (C)
Grass clippings	Pruning logs
Vegetables leftovers	Olive pomace
Coffee grounds	Grape stalk
Manures	Straws from cereals
Leachate	Sawdust
Sewage sludge	Dry leaves

There are several characteristics to be aware in order to use them as an organic amendment or as growing media such as the pH (that should be between 5.5 and 8.5) and the content of heavy metals (cadmium, lead, copper, chromium, mercury, nickel and zinc) (Brito, 2017; Ribeiro, *et al.*, 2009). The composts that are going to be tested in this experiment, are fertilizing materials that belong to group 5 – organic amendments - and are classified as Class I (can be used in agriculture) and Class IIA (can only be used in tree and shrub crops such as olive groves and vineyards, as well as in forest species) (Decreto Lei nº 113/2015 of June 15 of Ministério da Economia, 2015). The maximum allowed values of heavy metals for these classes are represented in Table 3.

Table 3. Maximum allowed values for the total content of heavy metals in organic compound by class (adapted from Sempiterno, 2016).

Parameter (mg kg^{-1} of dry matter)	**Class I**	**Class IIA**
Cadmium (Cd)	0.7	3.0

Lead (Pb)	100	300
Copper (Cu)	100	400
Chromium (Cr)	100	300
Mercury (Hg)	0.7	3.0
Nickel (Ni)	50	200
Zinc (Zn)	200	1000

In Portugal there are 23 infrastructures that make organic waste valorization of urban residues. The majority receives urban residues that are collected in an undifferentiated manner, with the exception of three infrastructures that select the urban residues that are going to be treated. One of these infrastructures is Algar® which is the only one that processes only green residues (APA, 2017).

In this experiment, one of the organic amendments that is going to be tested is a product obtained from composting solely green residues (vegetable waste from gardens, parks, forests or similar) (Decreto Lei nº 113/2015 of June 15 of Ministério da Economia, 2015) and the other is a result of composting sewage sludge and pine bark, obtained from a Waste-Water Treatment Plant.

2.4.1. Waste Water Treatment Plants

With the increase of the human population and the expansion of the urban areas, there is an augment of the quantity of domestic water supply and sewerage, thus a raise of municipal waste water. All the industry and human activities create effluents and, with the increase of the awareness of the present environmental issues, these waste products need to be disposed in a way that they do not represent a danger to human health or a problem to the environment (FAO, 2019). This is the main objective of a Waste-Water Treatment Plant - WWTP.

The wastewater goes through several processes (physical, chemical and biological) with the objective to clear them from pollutants (Hreiz *et al.*, 2015), resulting in a product called sewage sludge.

Sewage sludge is an inevitable product of the WWTP and it is composed of excess biomass that forms along the process of transforming the suspended particles, by the action of microorganisms, removal of organic matter and other nutrients (Águas do Algarve, 2017).

Sewage sludge is a product characterized by having a high content of organic matter, humidity and nutrients. If not well treated, the high content of organic matter can start the process of fermentation, in anabiosis conditions, releasing toxic gases to the atmosphere. Preferably, should be a stabilized product, to avoid this problem and to be safely applied to the soil (d'Azevedo, 2015). The other main problem of this type of waste is the presence of elements such as heavy metals and pathogens in their composition that can be harmful for the human health and the environment (Ribeiro, *et al.*, 2009; Mota & Mestre, 2007).

Finding a destiny to this type of waste is a problem that needs a sustainable solution since that around 57% of the sewage sludge produced in Portugal is dumped in landfills and 10%, of the remaining 47%, is destined for composting for later use in agriculture (APA, 2017).

2.5. The Cork Industry in Portugal

2.5.1. The cork oak in Portugal

Portugal is the world leader in the production of cork, corresponding to 49.5% of the world production, which is 100.000 t of cork produced per year (APCOR, 2016), and it is the export leader with a quota around 65% (APCOR, 2018).

The cork comes mainly from *Quercus suber* L. (family *Fagaceae*) that are cropped in a very particular agroforestry system criated by man, the “Montado” (WorldPress, 2019) or in a high cork oak density forest, that only exists in the Mediterranean region, Argelia, Morocco and in the south regions of the Iberic Peninsula.

This agroforestry system contributs to the preservation of the environment throught its fundamental role in the hydrological regulation, preservation of the soils and the biodiversity, and provides protection against the aeolic and hidrologic erosion (APCOR, 2018).

In Portugal, “Montado” is legaly protected which means that it is illegal to cut down cork oaks. This fact makes the *Quercus suber* the second forest species that occupies most area, around 23% of the national forest (*i. e*. 736.775 ha) (APCOR, 2016). This makes Portugal the mundial leader in cork export and the producer of bottle stoppers and other cork products (WorldPress, 2019) and consequently, it is necessary to treat the waste produced by the industrial processing of the cork.

2.5.2. The residues from the cork industry

Cork is a natural product that is extracted from the cork trees, every nine years. Its properties like porosity (more than 50% of its volume is air), impermeability to gases and water (due to the presence of suberin in the cellular walls), its high resistance to friction and excellent thermal and acoustic insulation capacity, makes this product a very versatile raw material (AMORIM, 2015).

Its main destination is the wine industry followed by the construction activity. From the industrial process, that the cork planks go through, there are three types of residues that are formed: “cork powder”, ADT granulate (hight density of “earth”) and cork “earth”.

“Cork powder” is a waste composed by cork particles with a dimension lower then 0.25 mm, that are not suitable for granulates. There are many types of cork powder and are composed of cork impurities and cork material powder that are produced throughout the various stages of cork processing. This waste product is used mostly as combustion fuel but it is also used in the production of linoleum and used as fillers, when mixed with glue, to produce worst quality cork stopper (Gil, 1997).

ADT granulate is a granulate that is not used in the production of cork stoppers. They are a subproduct of the cork griding for the production of agglomerates (Dinis, 2014). The particules dimension of this subproduct vary between 0.5 and 2 millimeteres and its bulk density is superior to cork. One of the characteristics of this material is its toxicity that can negatively influence the germination of seeds (Silva, 2018).

The cork “earth” is a residue of the bottle stoppers production and other products with lower quality. It results of the grinding of the back (a poor quality outer layer of the cork plank) and the inner layer (“belly” - the best quality part of the cork plank) of the cork planks. Due to the fact that these planks are more fibrous than other tissues, this residue presents a high density compared to other types of residues of this industry (Luís & Cortiço, 2009). This organic material has a significant amount of soil in its composition, from its various locals of origin and since it did not go through any form of processing, this residue has a seed bank with the various vegetative species that are present in their respective local of origin. Because there is no further utilization for it, it is responsibility of the company to pay for other companies to do the waste management of this product.

3. Materials and methods

3.1. Location of the study

The present study took place in the Chemical Agricultural Plant Nursery "Professor Boaventura de Azevedo" located in the Instituto Superior de Agronomia (ISA) Campus, University of Lisbon, in Tapada da Ajuda, in Portugal (Figure 7). The Nursery is an infra-structure constituted by two parts: a greenhouse and an open shelter (further details in INIAV, 2009). This experiment occurred in the greenhouse part of the Nursery (Figure 8).

Figure 7. The location of the Chemical Agricultural Plant Nursery "Professor Boaventura de Azevedo" (adapted from Google Earth, 2019).

Figure 8. Side view of the greenhouse where the study took place (adapted from INIAV, 2009).

3.2. Plant material

The two turf grass species used in this experiment were *Lolium perenne* cv. 'Benchmark' and *Agrostis stolonifera* cv. 'Penn-A4'. The seeds were provided by the company A. Pereia Jordão, Lda®, located in Maia, Porto.

The amounts of *L. perenne* and *A. stolonifera* seeds that were sowed were calculated according to the recommendation of the company that provided the seeds and according to the area of the pots (Table 4).

Table 4. The amount of seeds sowed per pot, for each turfgrass species

Grass Species	Recommendation (kg ha^{-1})	Amount seed per pot (g)	Number of seeds
L. perenne cv. 'Benchmark'	100	2.09	1209*
A. stolonifera cv. 'Penn-A4'	550	0.38	57000**

*Average counted number in 0.38 g of seeds.
**Estimated number of *Agrostis stolonifera* cv. 'Penn-A4' seeds per pot (Seeds, 2019).

3.3. Materials used in the sand-based rootzone mixture

The materials used in the rootzone mixture in this experiment were silica sand (**SS**), raised bog peat (**PT**), a residue from the cork industries: cork "earth" (**CE**) and two commercial organic composts: one made from green residues, Nutriverde Premium® (**NV**) and the other made from sewage sludge with pine bark, Naturanat® (**NA**) (Figure 9).

The physical characteristics, the proportion of the materials used in the different rootzone mixtures and the maximum particle size of the organic amendments allowed (6.4 millimeters) where chosen according to the USGA – United State Golf Association, recommendation for putting greens construction (USGA, 2018). The physical, chemical and physicochemical characteristics of the materials used are shown in Table 5 and the rootzone mixtures were analyzed at the end of the experiment.

3.3.1. Silica sand

The sand that is used in the rootzone mixture has to respect certain parameters such as grain size and the content of minerals that compose it (USGA, 2018).

The type of sand that was used in this experiment was silica sand S40/45® provided by the company Sifucel - Sílicas S.A® (Parapedra Group), located in Rio Maior.

77.6% of the grain size of this sand is 0.250 millimeters in diameter and 99.4% is composed of quartz (SiO_2). According to the USGA recommendations, the particle size of the sand classifies it as "medium sand" and it is accepted for a putting green rootzone mixture. The percentage of quartz makes it chemically inert thus resistant to chemical changing over time (USGA, 2018).

3.3.2. Raised bog peat

The brand of the peat used in the experiment is Floradur A block® and it was provided by the company Floragard - Vertiebs GMBH fur Gartenbau, located in Germany. This product comes from a natural source and it is composed by almost entirely undecomposedand very highly decomposed raised bog peat (H2 –H8 in the scale of von Post) (Table 1). Around 10% of this product is composed by light peat and 90% is composed by dark peat and it is a product free of weeds and pests.

This type of peat (dark peat) was recommended by professionals of the company for the purpose of this experiment. Regarding this product, the particles sizes are: 1% > 5mm; 27% > 2 mm; 41% > 1mm; 15% > 0.5 mm and 17% < 0.5 mm; meeting the USGA recommendations.

3.3.3. Naturanat®

Naturanat® is provided by the Services of Maia Municipality and it results in the co-composting of sewage sludge – a residue of Waste Waters Treatment Plant – with pine bark (50% - 50%). It is a stabilized product, being classified as class IIA quality for fertilizing materials, Group 5 – Organic Amendment (DGAE, 2015).

The particle sizes of this product are: 12% > 5mm; 40% > 2 mm; 21% > 1mm; 13% > 0.5 mm and 14% < 0.5 mm. Since 88% of the particle size is under the maximum particle size according to the USGA recommendations, this product was acceptable to be used as an organic amendment in the rootzone mixture.

3.3.4. Nutriverde Premium®

This product is obtained from the composting of solely green residues originated from urban, agriculture and gardening activities such as parks, gardens and golf courses. It is produced in the Compost Unit of Green Residues of the company Algar® (EGF Group), localized in São Brás de Alportel, in Algarve.

This is a class I quality compost (Group 5 – Organic Amendment), 100% natural organic compost that has been matured and therefore, stabilized and free of seeds and vegetative propagules. The texture of this product is very fine and the particle sizes are all under 5 mm: 3% > 2mm; 17% > 1 mm; 19% > 0.5 mm and 60% < 0.5 mm, making it suitable for being used in this experiment.

3.3.5. Cork "earth"

This product was provided by Equipar – Cork Industry, S.A., and this organic material is collected from several locations of the country. This material is a waste from the cork industries and it results mostly in the grinding of the dark part of the cork planks and it comes with a considerable amount of soil, from its local of origin.

The physical characteristic that indicated that it could be used in this experiment, as an organic amendment in the rootzone mixture, was size of the cork particles that are all under 5 mm: 17% > 2 mm; 29% > 1mm; 27% > 0.5 mm and 27% < 0.5 mm, meeting the USGA particle size recommendations for organic amendments used in the rootzone mixture.

Figure 9. The materials used in the root zone mixture: silica sand (1), peat (2), Naturanat® (3), Nutriverde Premium® (4) and cork “earth” (5).

Table 5. The costs of the organic amendments (per L and per ha), the bulk density, organic matter (OM), pH, electrical conductivity (EC), macronutrients (N, P, K, Ca, Mg, S), the micronutrients (Fe, Mn, B, Zn, Cu and Mo) and the heavy metals (Pb, Cr, Ni, Cd) of the materials used in the rootzone mixture: silica sand (SS), peat (PT), sewage sludge and pine bark (NA), green compost (NV) and cork “earth” (CE).

Parameter	**Unit**	**Silica sand** (SS)	**Peat** (PT)	**Sewage sludge and pine bark** (NA)	**Green compost** (NV)	**Cork "earth"** (CE)
Price per liter*	€	-	0.139	0.026	0.031	-
Costs per ha**	€	-	70980.00	13260.00	15806.70	-
Bulk density	kg m^{-3}	1550	433	608	689	383
Organic matter (OM)	%	0.02	29.86	39.79	33.59	71.48
pH (H_2O) (1:2.5)		7.9	4.19	4.15	8.98	5.99
Electrical conductivity (EC) (1:2)	mS cm^{-1}	0.01	0.63	0.66	1.46	0.47
Nitrogen (N)	mg kg^{-1}	-	2378.92	9120.56	9434.05	5379.42
Phosphorus (P_2O_5)	mg kg^{-1}	1	218.86	6823.61	4419.89	1030.80
Potassium (K_2O)	mg kg^{-1}	5	831.70	950.94	8855.18	6186.56
Calcium (Ca)	mg kg^{-1}	5	3191.56	6068.46	43391.83	17457.66
Magnesium (Mg)	mg kg^{-1}	-	507.70	680.74	3085.19	2304.29
Sulfur (S)	mg kg^{-1}	-	811.73	2347.92	2378.67	870.32
Sodium (Na)	mg kg^{-1}	-	150.15	188.16	1514.98	236.58
Iron (Fe)	mg kg^{-1}	0.6	262.94	1616.41	3237.19	2063.77
Manganese (Mn)	mg kg^{-1}	4.9	17.61	49.72	83.87	185.17

Boron (B)	mg kg^{-1}	0.06	<0,10	4.66	29.06	8.04
Zinc (Zn)	mg kg^{-1}	<0.1	1.99	425.49	56.21	9.36
Copper (Cu)	mg kg^{-1}	0.2	11.46	93.52	25.59	14.04
Molybdenum (Mo)	mg kg^{-1}	0	<0,1	0.92	<0,1	<0,1
Lead (Pb)	mg kg^{-1}	0	<0,1	6.94	2.92	<0,1
Chromium (Cr)	mg kg^{-1}	0	0.44	20.92	6.37	14.47
Nickel (Ni)	mg kg^{-1}	0	0.27	8.69	0.28	6.70
Cadmium (Cd)	mg kg^{-1}	0	<0,1	<0,1	<0,1	<0,1

* Value without VAT (value added tax) at the prevailing rate.
** Value calculated for one hectare, with a depth of 0.255 meters using 20% of organic amendment (510000 L).

3.4. Setup and assembly

In this study, four rootzone mixtures (sand mixed with: peat – PT, Naturanat® - NA, Nutriverde Premium® - NV, and cork "earth" – CE), two species of turfgrass (*Lolium perenne* cv. 'Benchmark' and *Agrostis stolonifera* cv. 'Penn-A4') and four replicates for future statistic analysis, were tested in order to compare and evaluate their performance in the growth of turfgrass.

32 *Kick-brauchkmann* pots where used for the development of this experiment plus 4 additional pots for weeds control, in a total of 36 pots. Pots were positioned in special pot holders, in blocks of four (with the four types of rootzone mixtures) distributed randomly, and placed over *Decauville* rails (Figure 10 and 11), that allows them to be moved in groups of four, to assure that all of the pots shared the same conditions, and inside and out of the greenhouse, in order to control the temperature and to avoid the rain over the plants, thus making it possible to control de irrigation (INIAV, 2009).

Four different rootzone mixtures were formulated and used to fill the pots, leaving a free edge of 1.5 centimeters height. Given that every organic amendment has different densities, resulting in mixtures with different porosities, this height was to guarantee that all the future shoot samples were made equally, between all the pots.

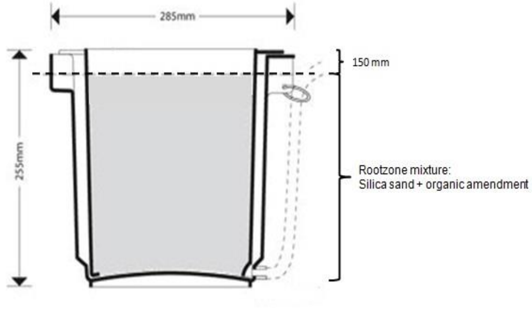


Figure 10.The representation of a *Kick-brauchkmann* pot, used in this experiment, with the total area occupied by the rootzone mixture (adapted from Stoma GmbH, 2017).

Figure 11. The 32 *Kick-brauchkmann* pots fully assembled, disposed over the *Decauville* rails.

From the 36 *Kick-brauchkmann* pots, 32 were used to produce turfgrass: 16 pots to produce *Lolium perenne* cv. “Benchmark” and 16 pots to produce *Agrostis stolonifera* cv. “Penn-A4” (Figure 12 and 13). The remaining four were used as a control treatment, for the weeds, after being used to determine the Maximum Water Holding Capacity (MWHC) of the rootzone mixtures.

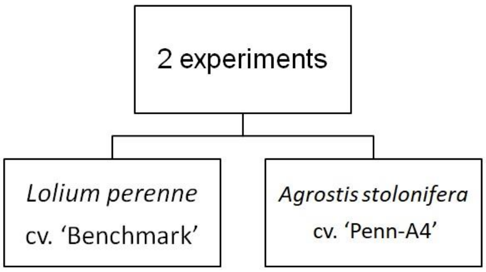


Figure 12. The two experiments done with the plant material used.

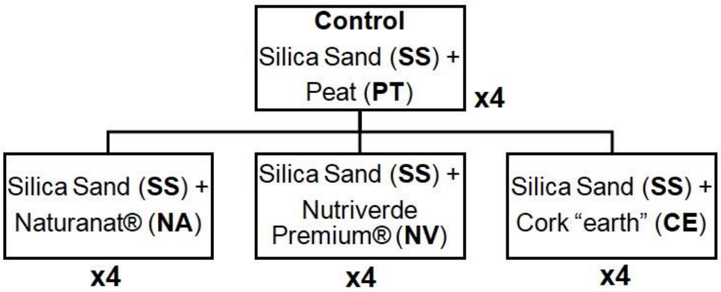


Figure 13. Representative scheme of the four rootzone mixtures (treatments), with the four repetitions, with peat (PT) as the control and the three organic amendments: Naturanat® (NA), Nutriverde Premium® (NV) and Cork “earth” (CE).

Each pot has 8 L volume capacity and a top area of 0.0380 m^2 (inner diameter of 0.22 m). For the sand-based root zone mixture, the silica sand and the organic amendments were mixed in an 80%-20% ratio (v v^{-1}) (Lewis *et al.*, 2010) respectively, *i. e.* 6.4 L of sand and 1.6 L of organic matter. Solid fertilizer was added to the mixture, before filling the pots.

3.5. Fertilization

The application of phosphorus (P_2O_5) and potassium (K_2O) was provided using a solid fertilizer (brand ADP Fertilizantes, S.A ®), with the amounts 0-20-17. Before adding 3.42 g of solid fertilizer in each pot (0.68 g of P_2O_5 and 0.58 g of K_2O), the solid fertilizer was previously ground and passed through a 2 mm sieve.

The application of nitrogen fertilizer was fractioned throughout the experiment (top-dressing). In each top-dressing, it was applied 5 mL of a solution of ammonium nitrate (NH_4NO_3), approximantely 0.10 g of N, diluted in 245 mL of distilled water per pots, in both turfgrasses (INIAP, 2006), according to the schedule present at Table 7.

3.6. Maximum water holding capacity of the rootzone mixtures and irrigation

To determine the maximum water holding capacity (MWHC), in four pots silica sand was mixed with one organic amendment, without the solid fertilizer and they were weighed to register their dry weight. After being irrigated at their full capacity, they were left without drainage and evaporation for 48 hours. The wet weight was registered after allowing drainage, for another 48 hours. The MWHC is calculated with the difference between the wet and the dry weight of the pots (adapted from Costa, 2004).

The irrigation management was made by weighting the pots twice a week, after germination, in order to verify the variations of the pots weight and irrigate when needed.

Shortly after sowing, the pots were irrigated to 70% of the MWHC and approximately 2 L of water were sprayed on top of the pots, 6 times a day (from May 20th to June 2nd), to maintain the sand surface moist. Until June 13th the pots were irrigated at their 70% of MWHC and once the plants had a considerable developed root system, the amount of irrigating water was 80% of the MWHC, until the end of the shoot harvesting, July 26th.

The change in the quantity of water for irrigation was due to the fact that both grasses had a considerable amount of biomass produced and therefore, the evapotranspiration increased, resulting in the need to augment the amount of irrigation water or the irrigation frequency. This was verified during the pot weighing process, throughout the experiment.

3.7. Sowing of seeds

The *L. perenne* cv. “Benchmark” certified seeds were covered with 0.589 kg of silica sand (one cm height), per pot, and the *A. stolonifera* cv. “Penn-A4” seeds were covered with 0.295 kg of silica sand (half a centimeter height), per pot.

The sowing was carried out at the May 15th and, until germination (May 20th), the pots remained outside the greenhouse, to keep them according to the environmental temperature (Table 6), and they were covered with a garden shade cloth, to avoid overheating of the sand surface (Figure 14), until germination.

Table 6. The average maximum and minimum air temperature (T) (°C), relative humidity (RH) (%) and global solar radiation (J m^{-2}) registered during the months in which the experiment was performed.

Months	**Average temperature** (T) (°C)		**Average relative humidity** (RH) (%)	**Average global solar radiation** (J m^{-2})
	Maximum	**Minimum**		
April	19.4	9.5	75	19870270
May	25.9	14.0	62	25768739
June	24.2	13.4	68	25844610
July	27.3	16.8	71	26024771
August	29.4	16.7	65	24731348

Figure 14. The pots covered with the shading cloth when the sowing took place (May 15th).

3.8. Shoot harvest and aerial biomass determination

The shoot harvesting was made using a pair of scissors and the plants were cut at the height of 1.5 centimeters. The first harvest of *L. perenne* occurred, approximately, three weeks after the sowing (June 7th) and the first harvest of *A. stolonifera* occurred four weeks after the sowing (June 14th). All the harvests were performed once a week, until the end of the experiment (July 26th).

After each shoot harvest, the samples were weighed, using a precision balance (0.01 g), to register the fresh weight, and weighed again to register the dry weight, after being dried at 65°C for 48 hours.

3.9. Maximum root length and root biomass

The root system is composed of main, seminal and adventitious roots. These last ones develop after the plant is established and are related to the success of the establishment of the plant (Newman & Moser, 1988).

The determination of the root biomass is a destructive process. At the end of the experiment, the pots were disassembled and the roots were washed in distilled water, to remove most of the organic particles possible. The roots were weighed in a precision balance (0.01g) after they were dried at 65°C for 72 hours, to register their dry matter.

To determine the average maximum root length, when the pots were disassembled, each grass mat cylinder was opened, lengthwise, and the total root length was measured in four locations: two at each end and two at the center of the cylinder (Figure 15).

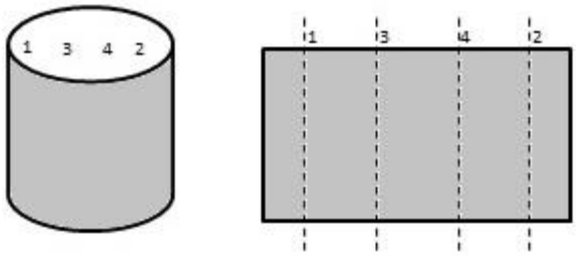


Figure 15. Representative scheme of the measuring locations of the maximum root length in each pot. The first (1) and second (2) measuring locations were in the peripheral site on the pot and the third (3) and fourth (4) were measuring locations in the inner part on the pots.

3.10. Determination of the number of plants per pot

To estimate the number of plants in each pot, when they were being disassembled, the turfgrass surface was divided in eight (α = 45°) and the plants were separated and counted (Figure 16). The total number of plants per pot was estimated by multiplying it by eight. The one eighth of the area, to count the plants, was chosen randomly.

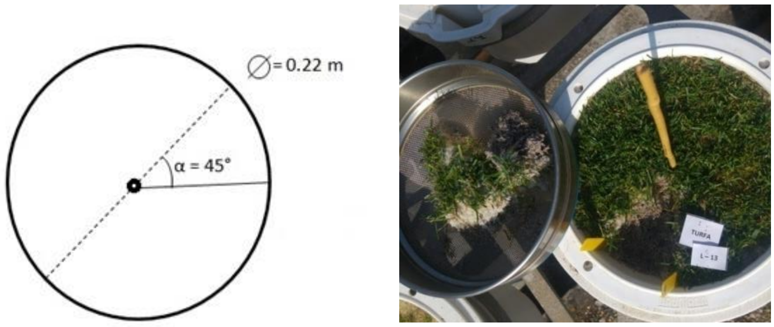


Figure 16. The grass mat surface division for the plant counting (left) and a random section removed, from the grass mat, to start counting plants (right).

3.11. Phenological states evaluation

As the tiller density is a very important factor in the uniformity of the turf grass, throughout of the development of the plants, their phenological state was determined based in the “A decimal code for the growth stages of cereals” (Zadoks *et al*., 1974).

Four plants were picked, randomly, in each type of rootzone mixture, three times during the experiment: in the begging, the middle and at the final stage.

3.12. Identification of the weeds

The taxonomical identification of the weeds was made between the 25th of May and the 22nd of September, *in situ*, with the collaboration of the Engineer Teresa Vasconcelos, a specialist in plant identification of the Instituto Superior de Agronomia, with resource to Caixinhas (2001) and Santo *et al.* (2014).

Weed biology, ecology and geographic distribution were based in the “Nova Flora de Portugal (Continente e Açores)” (Franco, 1971; 1984; Franco & Rocha Afonso, 1998).

3.13. Identification and fungi treatment

During the development of the experiment, the presence of two different species of fungi appeared in various pots.

To identify one of them, samples of the infected vegetative material were taken (June 21st) and isolated in Petri dishes with a 1% agarose (Difco^{TM}) growing medium, after being disinfected with a 1% sodium hypochlorite solution and washed with distilled water. The Petri dishes were placed in a moist chamber, at room temperature and the fungi structures were analyzed one week after the sampling date.

The fungus structures and the conidia morphology were examined under an electronic binocular magnifying glass (Leica®, model MZ 12.5) and the fungus species was identified with resource to Sivanesan (1987). Both grass species were treated twice, with a week interval between pulverizations (July 1st and July 8th) with the fungicide a.i. azoxystrobin (Ortiva®) and in the first pulverization, an insecticide was also added to the pulverization solution, a.i. deltamethrin (Decis®).

The identification of the second fungus species was based on the observation of its macromorphological characteristics, by photography.

3.14. Plant analysis

The macro and micro elements (K, P, Mg, Ca, S, Fe, Cu, Zn, Mn, B and Na) analysis was performed by *aqua regia* digestion, according to the European Norm EN 13650 (CEN, 2001) adapted, and quantified by an Inductively Coupled Plasma Optical Emission Spectrometry, ICP – OES (ThermoFisher Scientific®, model iCAP7000 series).

The nitrogen was determined by Kjeldahl digestion method, with sulfuric acid (H_2SO_4) and the catalyst, selenium (Horneck & Miller, 1998), and quantified by Segmented Flow Analysis (SFA) (Skalar®, model SAN plus systems) according to the Berthelot method (Houba *et al.*, 1989).

For the plant analysis, the biomass of the 7 harvest was mixed in two groups. Since one of the objectives of this experiment was to observe the organic amendments nitrogen residual effect in the plants growth, following groups were established: for the *L. perenne,* group **A** is the set of samples from the first harvest to the forth and the group **B** is the remaining three harvests. For *A. stolonifera*, group **A** is the set of the first to the third harvests and group **B** is the remaining four harvests (Table 7).

Table 7. Timing of the fertilizations and the harvests carried throughout the experiment, of *Lolium perenne* cv. 'Benchmark' and *Agrostis stolonifera* cv. 'Penn-A4'.

	***Lolium perenne* cv. 'Benchmark'**		***Agrostis stolonifera* cv. 'Penn-A4'**	
Date	**Practices**	**Harvest group**	**Practices**	**Harvest group**
May 15th	Sowing	-	Sowing	-
May 20th	Germination	-	-	-
May 22nd	-	-	Germination	-
June 7th	1st harvest N fertilization	A	- -	- -
June 14th	2nd harvest	A	1st harvest N fertilization	A

June 21st	3rd harvest	A	2nd harvest	A
June 28th	4th harvest N fertilization	A	3rd harvest	A
July 6th	5th harvest N fertilization	B	4th harvest N fertilization	B
July 12th	6th harvest N fertilization	B	5th harvest N fertilization	B
July 19th	7th harvest -	B	6th harvest N fertilization	B
July 26th	-	-	7th harvest	B

3.15. Rootzone mixture sampling and preparation at the end of the experiment

After disassembling of the pots, rootzone mixture samples were collected and allowed to dry at room temperature in the Nurserys open shelter section, during August, to allow all the moisture to evaporate. The rootzone mixtures were then crushed using a mortar and pestle, and passed through a 2 mm sieve in order to reduce the particle size of the organic amendments and to make the rootzone mixtures more uniform, to facilitate the future analyses.

3.16. Chemical and physicochemical characterization of the rootzone mixtures

The pH (Thermo Electron Corporation®, model Orion 3 Star) and the electrical conductivity (EC) (Thermo Scientific®, model Orion Star A212) were measured in a 1:2.5 and a 1.2 (w v^{-1}) soil : water suspension, according to the European Norms EN 13037 (CEN, 1999a) and EN 13038 (CEN, 1999b) respectively.

The total organic matter (OM) was determined by dry-combustion method (Tiessen & Moir, 1993) where the samples were heated at 1200°C and the CO_2 levels were analyzed by an Analytik Jena (TOC®, model MultiEA 4000).

The extractable phosphorus and potassium were extracted using an Egner-Rihem solution (Egner H., 1960) and were quantified by an Inductively Coupled Plasma Optical Emission Spectrometry, ICP - OES (ThermoFisher Scientific®, model iCAP 7000 series).

The exchangeable bases (Ca, Mg, Na, K) were extracted using 1 mol^{-1} L ammonium acetate solution ($C_2H_7NO_2$) (Schollenberger & Simon, 1945) and were quantified by an Inductively Coupled Plasma Optical Emission Spectrometry, ICP - OES (ThermoFisher Scientific®, model iCAP 7000 series).

The micro elements (Fe, Cu, Zn and Mn) were extracted with a Lakanen solution (Lakanen & Ervio, 1971) and quantified by an Inductively Coupled Plasma Optical Emission Spectrometry, ICP - OES (ThermoFisher Scientific®, model iCAP 7000 series).

To analyze the exchangeable acidity, 1 mol L^{-1} potassium chloride (KCl) extraction was performed and quantified by an acid base titration method (Roades, 1982), using 0.043 mol L^{-1} sodium hydroxide solution (NaOH) and phenolphthalein as the indicator (Metrohm®, model 775 Dosimat).

3.17. Statistic analysis and data processing

The results of the aerial and root biomass production, the total root length, the number of seeds per pots and the chemical and physicochemical characteristics of the rootzone mixtures were obtained with one-way analyses of variance (ANOVA). The statistical significance of results between the treatments (rootzone mixtures) and the plants groups (A and B) was obtained with two way analyses of variance (ANOVA). The differences of means were compared using LSD Fisher's least significant difference, with a significance level 5% ($P \leq 0.05$) (Montgomery, 1991). The results were organized according to the treatments used and the program that was used to do the statistic analysis was the “Statistix 9” program.

4. Results

4.1. Plant development

The results of the aerial biomass production were separated in two groups, **A** and **B**, for both grass species.

Regarding *Lolium perenne* cv. 'Benchmark', group **A** is the sum of the shoots from the first to the fourth harvests (V1 - V4) - where the plants were fertilized only once - and group **B** is the sum of the shoots from the fifth to the seventh harvests (V5 - V7) - where the plants were fertilized weekly.

The same was done for *Agrostis stolonifera* cv. 'Penn-A4': group **A** is the sum of the shoots from the first to the third harvests (V1 – V3) - where the plants were fertilized only once - and group **B** is the sum of the shoots from the fourth to the seventh harvests (V4 – V7) - where the plants were fertilized weekly.

4.1.1. Aerial biomass production and the number of plants per pot

About *L. perenne*, in the first shoot harvest (V1), the rootzone mixture that had the highest amount of dry matter production was the rootzone mixture with peat (PT), followed by the rootzone mixture with sewage sludge and pine bark (NA). Until the third shoot harvest (V3), the production of dry matter in the rootzone mixture PT, decreased and became inferior to the rootzone mixture NA and the rootzone mixture with green compost (NV). After the fourth shoot harvest (V4), when the plants were fertilized with nitrogen once a week, the production of aerial biomass increased, in all rootzone mixtures but in PT (the biomass production did not followed the same obtained from NA and NV) (Table 8).

Table 8. The amounts of dry matter obtained from shoots harvesting of *Lolium perenne* cv. 'Benchmark' (g), performed every week (V1 – V7) during the experiment (from June 7th to July 19th), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**V1** (g)	**V2** (g)	**V3** (g)	**V4** (g)	**V5** (g)	**V6** (g)	**V7** (g)
PT	2.03 **a**	0.99 **a**	0.89 **b**	0.46 **b**	0.76 **b**	0.75 **b**	1.09 **b**
NA	1.73 **a**	1.41 **a**	1.41 **a**	0.87 **a**	1.23 **a**	1.28 **a**	1.63 **a**
NV	0.85 **b**	1.02 **a**	1.43 **a**	0.90 **a**	1.36 **a**	1.35 **a**	1.59 **a**
CE	0.22 **c**	0.05 **b**	0.45 **c**	0.31 **b**	0.57 **b**	0.56 **c**	0.88 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p= 0.05).

In Table 9, by comparing the sum of the dry matter in group A (V1 to V4), the rootzone mixture NA had the higher amount of biomass produced, followed by the rootzone mixture PT. In group B (V5 to V7) the rootzone mixtures NV and NA had the higher amounts of biomass produced. At the end of the experiment, comparing the total amount of biomass produced in each rootzone mixture (A+B), NA and NV surpassed the production of PT and the rootzone mixture with cork "earth" (CE) had the lower amount of plant dry matter (Figure 17).

Table 9. The sum of the amounts of dry matter obtained from the shoot harvesting of *Lolium perenne* cv. 'Benchmark' (g), in group A (V1 – V4), group B (V5 - V7), and the total amount of dry matter produced (A+B), in the rootozone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**Total (A)** (g)	**Total (B)** (g)	**Total (A+B)** (g)
PT	4.38 **ab**	2.61 **b**	6.99 **b**
NA	5.42 **a**	4.14 **a**	9.56 **a**
NV	4.19 **b**	4.30 **a**	8.49 **a**
CE	1.02 **c**	2.01 **c**	3.03 **c**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p= 0.05).

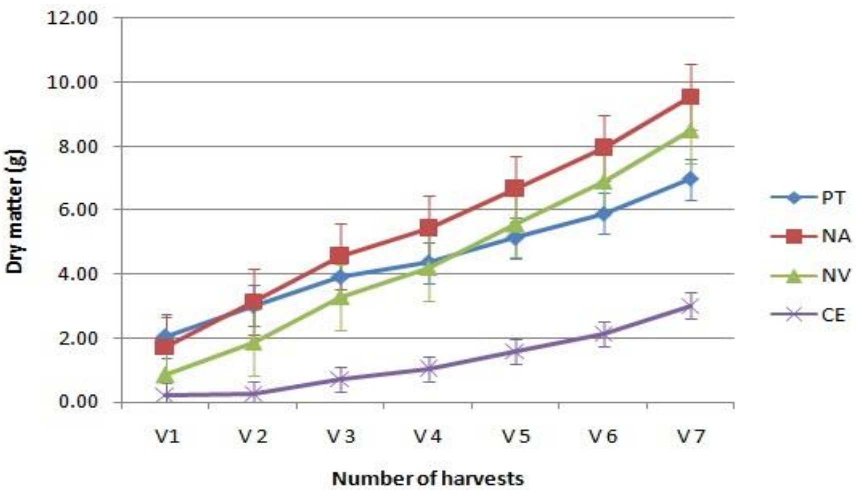


Figure 17. Dry aerial biomass accumulation produced by *Lolium perenne* cv. 'Benchmark' (g) obtained from the shoot harvests performed each week (V1 – V7), in the rootozone mixture: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Regarding *Agrostis stolonifera* cv. 'Penn-A4', in the first shoot harvest (V1), the rootzone mixture that had the highest amount of dry matter was the rootzone mixture with peat (PT), followed by the rootzone mixture NA (sewage sludge and pine bark compost). After the second shoot harvest (V2), the amount of biomass in PT decreased, while kept increasing in the rootzone mixtures NA and NV. In the third shoot harvest (V3), rootzone mixtures NA and NV exceeded biomass production by comparison with PT. When the regular nitrogen fertilization began (V4), the biomass production increased in all treatments being higher in the rootzone mixtures NA and NV (Table 10).

Table 10. The amounts of dry matter obtained from shoots harvesting of *Agrostis stolonifera* cv. 'Penn-A4' (g), performed every week (V1 – V7) during the experiment (from June 14th to July 26th), in the rootozone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	V1 (g)	V2 (g)	V3 (g)	V4 (g)	V5 (g)	V6 (g)	V7 (g)
PT	1.66 **a**	1.62 **a**	0.81 **c**	1.08 **b**	0.80 b	1.68 **b**	1.53 **a**
NA	0.88 **b**	1.80**a**	1.46 **a**	1.83 **a**	1.32 a	1.95 **a**	1.65 **a**
NV	0.42 **c**	0.99 **b**	1.24 **b**	1.67 **a**	1.66 **a**	2.05 **a**	1.79 **a**
CE	0.00 **d**	0.00 **c**	0.09 **d**	0.38 **c**	0.48 **c**	0.77 **c**	0.93 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p= 0.05).

In Table 11, by comparing the sum of the dry matter in group A (V1 to V3), there were no significant differences between the treatments PT and NA. In group B (V4 to V7), there were no significant differences between the treatments NA and NV. These treatments had the higher amounts of biomass produced. By comparing the total biomass produced in both groups (A+B), the rootzone mixture NA had the highest amount of biomass produced, followed by the rootzone mixtures NV and PT whose treatments had no significant differences (Figure 18). The rootzone mixture CE had the lowest amounts of dry matter produced since not enough biomass was produced in the first couple of weeks (V1 and V2) to allow a shoot harvest (Table 10).

Table 11. The sum of the amounts of dry matter obtained from the shoot harvesting of *Agrostis stolonifera* cv. 'Penn-A4' (g), in group A (V1 – V3), group B (V4 - V7), and the total amount of dry matter produced (A+B), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Total (A) (g)	Total (B) (g)	Total (A+B) (g)
PT	4.09 **a**	5.08 **b**	9.17 **b**
NA	4.14 **a**	6.75 **a**	10.89 **a**
NV	2.65 **b**	6.65 **a**	9.29 **b**
CE	0.09 **c**	2.55 **c**	2.64 **c**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p= 0.05).

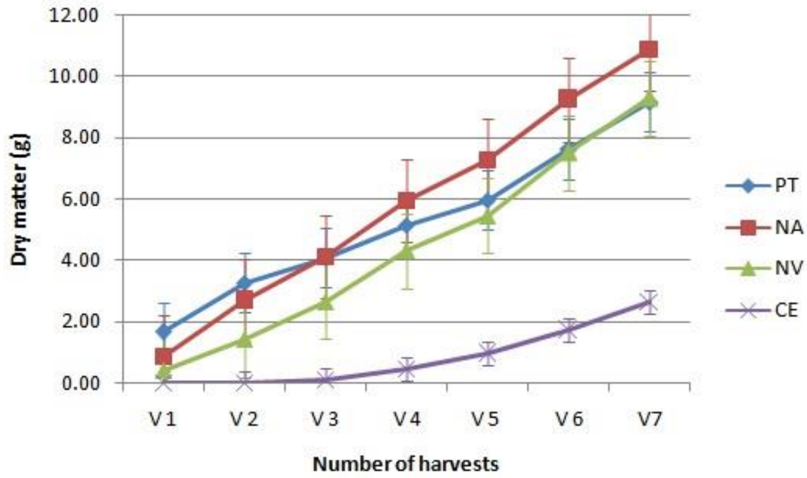


Figure 18. Dry aerial biomass accumulation produced by *Agrostis stolonifera* cv. ‘Penn-A4’ (g) obtained from the shoot harvests performed each week (V1 – V7), in each rootzone mixture: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE).

After counting the plants in the randomly chosen area of the surface of each pot, and estimating the total amount of plants in each one, the results obtained from *L. perenne* were consistent with those from *A. stolonifera*, which were as follows: there were no significant differences of the estimated number of plants, per pot, between treatments (Table 12).

Table 12. The estimated total number of plants, per pot, of *Lolium perenne* cv. ‘Benchmark’ and *Agrostis stolonifera* cv. ‘Penn-A4’, at the end of the experiment, in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE).

Rootzone mixture	***L. perenne* cv. ‘Benchmark’**	***A. stolonifera* cv. ‘Penn-A4’**
PT	816 **a**	2892 **a**
NA	956 **a**	1896 **a**
NV	934 **a**	2170 **a**
CE	1066 **a**	2646 **a**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).

4.1.2. Phenological states evaluation of *Lolium perenne* cv. ‘Benchmark’ and *Agrostis stolonifera* cv. ‘Penn-A4’

Regarding *L. perenne* cv. ‘Benchmark’, the evolution of the plants in the rootzone mixtures PT, NA and NV was very similar although the tillering was higher in the plants that developed in NA and NV (Tables 13, 14 and 15). The rootzone mixture CE showed the least plant development and the tillering was nonexistent (Figure 19).

Table 13. The decimal code for the phenological stage of *Lolium perenne* cv. 'Benchmark', in the beginning of the experiment (June 6th), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Zadoks growth stage	Development stage of main culm
PT	1.14	Four leaves expanded
	2.20	Primary branch differentiation
NA	1.14	Four leaves expanded
	2.21	Primary branch differentiation with one tiller
NV	1.15	Five leaves expanded
	2.20	Primary branch differentiation
CE	1.13	Three leaves expanded
	2.20	Primary branch differentiation

Table 14. The decimal code for the phenological stage of *Lolium perenne* cv. 'Benchmark', in the middle of the experiment (July 9th), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Zadoks growth stage	Development stage of main culm
PT	1.15	Five leaves expanded
	2.22	Primary branch differentiation with two tillers
NA	1.15	Five leaves expanded
	2.22	Primary branch differentiation with two tillers
NV	1.15	Five leaves expanded
	2.22	Primary branch differentiation with two tillers
CE	1.15	Five leaves expanded
	2.20	Primary branch differentiation

Table 15. The decimal code for the phenological stage of *Lolium perenne* cv. 'Benchmark', at the final stage of the experiment (July 17th), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Zadoks growth stage	Development stage of main culm
PT	1.15	Five leaves expanded
	2.22	Primary branch differentiation with two tillers
NA	1.15	Five leaves expanded
	2.23	Primary branch differentiation with three tillers
NV	1.16	Six leaves expanded
	2.24	Primary branch differentiation with four tillers
CE	1.16	Six leaves expanded
	2.20	Primary branch differentiation

Figure 19. The phenological stage of random plants of *Lolium perenne* cv. 'Benchmark' selected from each rootzone mixture, at the end of the experiment (July 17^{th}): 1) peat (PT); 2) sewage sludge and pine bark compost (NA); 3) green compost (NV) and 4) cork "earth" (CE).

Regarding *A. stolonifera* cv. 'Penn-A4', the plants that grew in the rootzone mixture PT presented more leaves expanded, but the plants that grew in the rootzone mixture NA, had more tillering (Tables 16, 17 and 18). The results obtained from the rootzone mixture CE were the same as those of *L. perenne* (Figure 20).

Table 16. The decimal code for the phenological stage of *Agrostis stolonifera* cv. 'Penn-A4', in the beginning of the experiment (June 12^{th}), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Zadoks growth stage	Development stage of main culm
PT	1.15	Five leaves expanded
	2.22	Primary branch differentiation with two tillers
NA	1.13	Three leaves expanded
	2.21	Primary branch differentiation with one tiller
NV	1.13	Three leaves expanded
	2.21	Primary branch differentiation with one tiller
CE	1.12	Two leaves expanded
	2.20	Primary branch differentiation

Table 17. The decimal code for the phenological stage of *Agrostis stolonifera* cv. 'Penn-A4', in the middle of the experiment (July 9^{th}), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Zadoks growth stage	Development stage of main culm
PT	1.15	Five leaves expanded
	2.23	Primary branch differentiation with three tillers
NA	1.13	Three leaves expanded
	2.23	Primary branch differentiation with three tillers
NV	1.14	Four leaves expanded
	2.22	Primary branch differentiation with two tillers
CE	1.12	Two leaves expanded
	2.20	Primary branch differentiation

Table 18. The decimal code for the phenological stage of *Agrostis stolonifera* cv. 'Penn-A4', at the final stage of the experiment (July 25th), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	Zadoks growth stage	Development stage of main culm
PT	1.16	Six leaves expanded
	2.23	Primary branch differentiation with three tillers
NA	1.17	Seven leaves expanded
	2.24	Primary branch differentiation with four tillers
NV	1.15	Five leaves expanded
	2.24	Primary branch differentiation with four tillers
CE	1.14	Four leaves expanded
	2.20	Primary branch differentiation

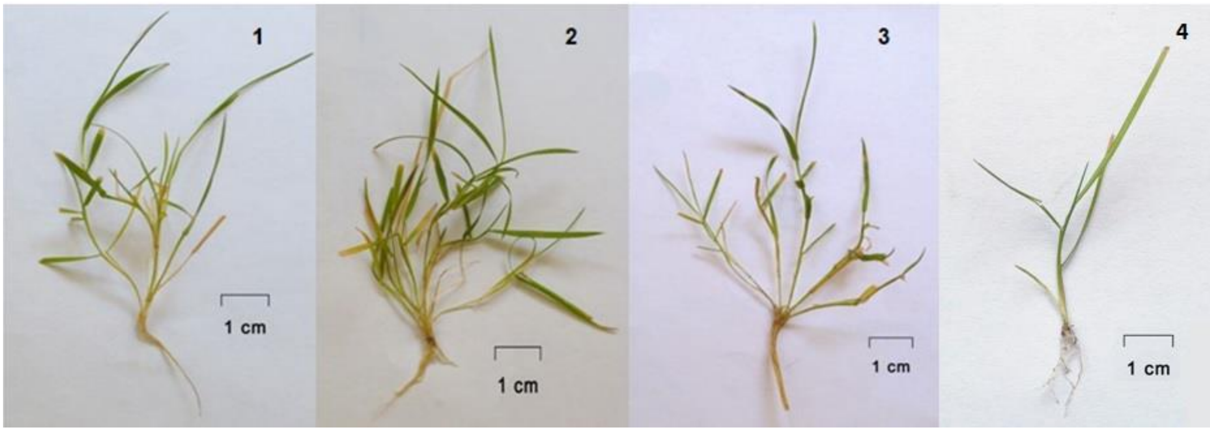


Figure 20. The phenological stage of random plants of *Agrostis stolonifera* cv. 'Penn-A4' selected from each rootzone mixture, at the end of the experiment (July 25th): 1) peat (PT); 2) sewage sludge and pine bark compost (NA); 3) green compost (NV) and 4) cork "earth" (CE).

4.1.3. Maximum root length and root biomass

For both grasses, there were very significant differences in the amounts of root biomass produced and in the maximum root lengths, between treatments. The plants that grew in the rootzone mixture with peat (PT) presented the highest amounts of root biomass produced and largest maximum root length. There were no significant differences of the root biomass produced by the plants that grew in the treatments NA (sewage sludge and pine bark compost), NV (green compost) and CE (cork "earth") (Table 19).

Regarding the maximum root length of *L. perenne*, there were no significant differences in the treatments NV and CE. However it was visible that, while disassembling the pots, the root of the plants that grew in NV was slightly shorter than the rootzone mixture CE (Figure 21). This was also perceptible in the pots disassemble with *A. stolonifera* (Figure 22).

Table 19. The average amounts of root biomass (g) and the respective maximum root length (m), in *Lolium perenne* cv. ‘Benchmark’ and *Agrostis stolonifera* cv. ‘Penn-A4’, produced in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE).

	***L. perenne* cv. ‘Benchmark’**		***A. stolonifera* cv. ‘Penn-A4’**	
Rootzone mixture	**Root biomass** (g)	**Maximum root lenght** (m)	**Root biomass** (g)	**Maximum root lenght** (m)
PT	24.92 **a**	0.43 **a**	14.50 **a**	0.43 **a**
NA	14.35 **b**	0.32 **b**	9.21 **b**	0.31 **b**
NV	16.40 **b**	0.19 **c**	8.62 **b**	0.16 **d**
CE	16.35 **b**	0.21 **c**	8.93 **b**	0.24 **c**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p= 0.05).

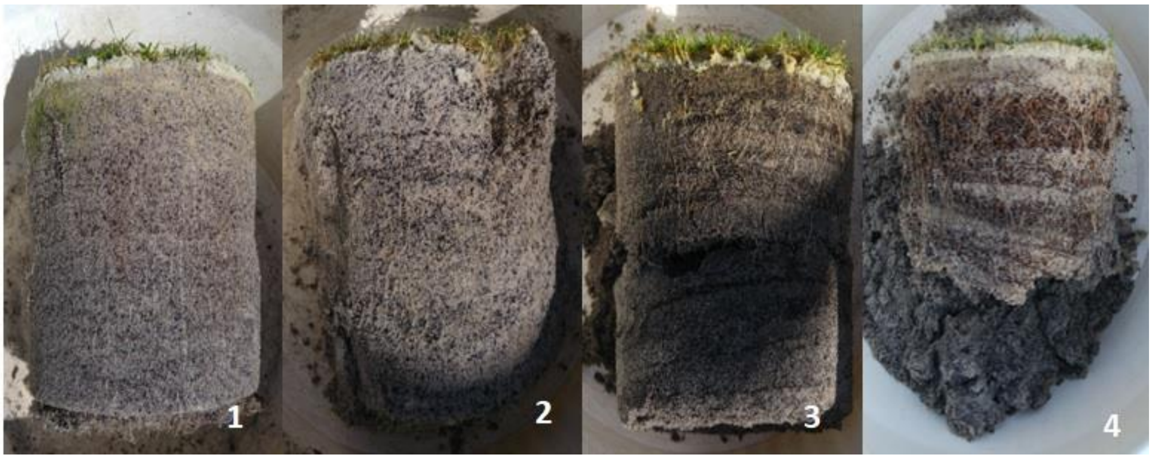


Figure 21. The maximum root length of *Lolium perenne* cv. ‘Benchmark’ observed, when the pots disassemble took place (July 24th), in the rootozone mixtures: 1) peat (PT); 2) sewage sludge and pine bark compost (NA); 3) green compost (NV) and 4) cork “earth” (CE).

Figure 22. The maximum root length of *Agrostis stolonifera* cv. ‘Penn-A4’ observed, when the pots disassemble took place (July 29th), in the rootzone mixtures: 1) peat (PT); 2) sewage sludge and pine bark compost (NA); 3) green compost (NV) and 4) cork “earth” (CE).

4.2. Chemical and physicochemical characteristics of the rootzone mixtures at the end of the experiment

4.2.1. *Lolium perenne* cv. 'Benchmark'

The rootzone mixture that presented the lowest pH was the rootzone mixture composed by sewage sludge and pine bark compost (NA), and the rootzone mixture that presented the highest pH was the rootzone mixture composed by green compost (NV). However none of the values of the four treatments are within the recommended range for turfgrass.

The electrical conductivity (EC) of the rootzone mixture with peat (PT) was the lowest and the NV was the highest, but none are considerate saline rootzone mixtures.

The results of the exchangeable acidity meet the results obtained from pH of the four treatments. The rootzone mixture NA had the lowest pH and the highest exchangeable acidity value. The rootzone mixture PT was the second with the higher exchangeable acidity value and the differences between the treatments NV and with cork "earth" (CE) did not differ significantly.

Considering the organic matter (OM) content, in the rootzone mixtures, there were very significant differences between treatments. The rootzone mixtures NA and NV were the ones with the higher OM values (Table 20).

Table 20. The average pH value in a misture:water suspension (w v^{-1}), electrical conductivity (EC - mS cm^{-1}), exchangeable acidity (cmol(+) kg^{-1}), organic matter (OM) (%), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	pH	EC (mS cm^{-1})	Exchangeable acidity	OM (%)
PT	4.90 **c**	0.09 **c**	0.20 **b**	0.91 **c**
NA	4.33 **d**	0.24 **b**	0.41 **a**	2.27 **ab**
NV	8.78 **a**	0.56 **a**	0.11 **c**	2.94 **a**
CE	7.10 **b**	0.15 **bc**	0.09 **c**	1.61 **bc**
Acceptable range	6.0 – 6.5*	< 0.40 – > 3.20*	-	-

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).
*Acceptable range of pH and EC according to INIAP (2006).

Regarding the extractable phosphorus (P_2O_5) and the extractable potassium (K_2O) in the rootzone mixtures, there were highly significant differences between treatments. The rootzone mixtures NA and NV had the higher amount of P_2O_5 and the rootzone mixtures PT and CE had the lowest amounts. The rootzone mixtures NV had the highest amount of extractable K_2O, followed by CE. The rootzone mixtures PT and NA had the lowest amounts, and there were no significant differences between this last two treatments (Table 21).

Table 21. Average extractable phosphorus (P_2O_5) and extractable potassium (K_2O) contents (mg per kg of rootzone mixture), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**P_2O_5** (mg kg^{-1})	**K_2O** (mg kg^{-1})
PT	21.49 **b**	6.12 **c**
NA	356.19 **a**	14.46 **c**
NV	268.43 **a**	414.34 **a**
CE	37.22 **b**	106.04 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

About the exchangeable bases (Table 22), there were higly significant differences between treatments. The rootzone mixture NV presented the highest amount of all exchangeable bases, followed by the rootzone mixture CE. The rootzone mixture PT had the lowest amounts of exchangeable bases.

Table 22. Average exchangeable bases contents (calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K)) (cmol(+) per kg of rootzone mixture), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**Ca** (cmol(+) kg^{-1})	**Mg** (cmol(+) kg^{-1})	**Na** (cmol(+) kg^{-1})	**K** (cmol(+) kg^{-1})
PT	0.59 **c**	0.14 **b**	0.008**b**	0.01 **c**
NA	1.90 **b**	0.31 **b**	0.02 **b**	0.03 **c**
NV	9.95 **a**	1.43 **a**	0.31 **a**	0.88 **a**
CE	1.49 **bc**	0.24 **b**	0.02 **b**	0.23 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

Concerning the extractable micronutrients in the rootzone mixtures (Table 23), there were highly significant differences between treatments. The rootzone mixture NA had the highest amounts of copper (Cu) and zinc (Zn) and the rootzone mixture NV had the highest amounts of iron (Fe) and manganese (Mn). Regarding Mn, there were no significant differences between the treatments NV and CE. The rootzone mixture PT had the lowest amounts of all nutrients with the exception of Zn, whose result had no significant differences between the treatments NV and CE.

Table 23. The average exctratable iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) contents in the rootzone mixtures (mg per kg of rootzone mixture) in each rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**Fe** (mg kg^{-1})	**Cu** (mg kg^{-1})	**Zn** (mg kg^{-1})	**Mn** (mg kg^{-1})
PT	9.39 **c**	0.26 **c**	1.15 **b**	1.54 **c**
NA	45.61 **b**	2.91 **a**	25.22 **a**	5.08 **b**
NV	101.32 **a**	1.07 **b**	3.19 **b**	8.85 **a**

CE	17.02 **c**	0.31 **c**	0.76 **b**	9.04 **a**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).

4.1.2. *Agrostis stolonifera* cv. 'Penn-A4'

Concerning the pH of the rootzone mixtures, there were highly significant differences between treatments. The rootzone mixture with green compost (NV) had the higher pH and the rootzone mixture that presented the lowest pH value was the rootzone mixture composed by sewage sludge and pine bark compost (NA). None of the values are within the recommended range for turfgrass.

Regarding the electrical conductivity (EC), the rootzone mixture NV had the highest value and between the treatments PT, NA and CE there were no significant differences. None are considerate saline rootzone mixtures.

The results of the exchangeable acidity meets the results obtained from the pH of the rootzone mixtures with the exception of rootzone mixture NV and the rootzone mixture with cork "earth" (CE), whose results had no significant differences between this two treatments. The rootzone mixture NA had the highest exchangeable acidity followed by the rootzone mixture PT.

The rootzone mixture PT was the one with the lowest amount of organic matter (OM) and there were no significant differences between the treatments NA, NV and CE (Table 24).

Table 24. The average pH value in a misture:water suspension (w v^{-1}), electrical conductivity (EC - mS cm^{-1}), exchangeable acidity (cmol(+) kg^{-1}), organic matter (OM) (%), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	pH	EC (mS cm^{-1})	Exchangeable acidity	OM (%)
PT	4.36 **c**	0.08 **b**	0.21 **b**	0.93 **b**
NA	4.25 **d**	0.20 **b**	0.38 **a**	2.26 **a**
NV	8.50 **a**	0.56 **a**	0.09 **c**	2.76 **a**
CE	6.42 **b**	0.13 **b**	0.08 **c**	2.01 **a**
Acceptable range*	6.0 – 6.5*	< 0.40 – > 3.20*	-	-

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).
*Acceptable range of pH and EC according to INIAP (2006).

Regarding the extractable phosphorus (P_2O_5) and the extractable potassium (K_2O) in the rootzone mixtures (Table 25), the differences between treatments were highly significant. The rootzone mixtures NV and NA had the highest amount of extractable P_2O_5, whose differences did not differ significantly. The rootzone mixture that had the highest amount of extractable K_2O was the rootzone mixture NV followed by the rootzone mixture CE. The rootzone mixture PT had the lowest amounts of extractable phosphorus and potassium.

Table 25. Average extractable phosphorus (P_2O_5) and extractable potassium (K_2O) contents (mg per kg of rootzone mixture), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**P_2O_5** (mg kg^{-1})	**K_2O** (mg kg^{-1})
PT	23.89 **b**	9.26 **c**
NA	259.45 **a**	12.63 **c**
NV	286.52 **a**	452.97 **a**
CE	46.19 **b**	222.57 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).

Regarding the exchangeable bases, the rootzone mixture NV was the one with the highest amounts of all the exchangeable bases. Regarding the other treatments, there were no significant differences between them, with the exception of the treatment CE that had the second higher value of exchangeable potassium (Table 26).

Table 26. Average extractable bases contents (calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K)) (cmol(+) per kg of rootzone mixture), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**Ca** (cmol(+) kg^{-1})	**Mg** (cmol(+) kg^{-1})	**Na** (cmol(+) kg^{-1})	**K** (cmol(+) kg^{-1})
PT	0.87 **b**	0.17 **b**	0.02 **b**	0.02 **c**
NA	1.25 **b**	0.18 **b**	0.02 **b**	0.03 **c**
NV	12.19 **a**	1.66 **a**	0.34 **a**	0.96 **a**
CE	1.99 **b**	0.27 **b**	0.01 **b**	0.47 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).

Regarding the extractable micronutrients (Table 27), there were highly significant differences between treatments. The rootzone mixture PT had the lowest amounts of all micronutrients. The rootzone mixture NA had the higher amounts of copper (Cu) and zinc (Zn), and the rootzone mixture NV had the higher amounts of iron (Fe) and manganese (Mn).

Table 27. The average exctractable iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) contents (mg per kg of rootzone mixture), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Rootzone mixture	**Fe** (mg kg^{-1})	**Cu** (mg kg^{-1})	**Zn** (mg kg^{-1})	**Mn** (mg kg^{-1})
PT	9.80 **c**	0.20 **c**	0.53 **c**	4.36 **d**
NA	42.65 **ab**	2.43 **a**	22.16 **a**	6.68 **c**

NV	58.51 **a**	1.31 **b**	4.29 **b**	14.06 **a**
CE	22.59 **bc**	0.35 **c**	1.29 **bc**	12.01 **b**

In each column, the treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

4.3. Mineral composition of the plants

The results of the mineral composition were separated in two groups, **A** and **B**, for both grass species.

About *Lolium perenne* cv. 'Benchmark', group **A** are the shoots from the first to the fourth harvests (V1 – V4), where the plants were fertilized only once, and group **B** are the shoots from the fifth to the seventh harvests (V5 – V7), where the plants were fertilized weekly.

The same was done for *Agrostis stolonifera* cv. 'Penn-A', group **A** are the shoots from the first to the third harvests (V1 – V3), where the plants were fertilized only once, and group **B** are the shoots from the fourth to the seventh harvests (V4 – V7), where the plants were fertilized weekly (Table 7).

4.3.1. *Lolium perenne* cv. 'Benchmark'

Regarding the nitrogen (N) content in the aerial biomass of the plants, there were no significant differences between the interaction of the two groups (A and B) and the four rootzone mixtures [peat (PT), sewage sludge and pike bark compost (NA), green compost (NV) and cork "earth" (CE)] (p = 0.157). Comparing both groups, the plants from group B had a higher amount of N in all four treatments and the rootzone mixture CE had the lowest amount of N both in group A and B (Figure 23).

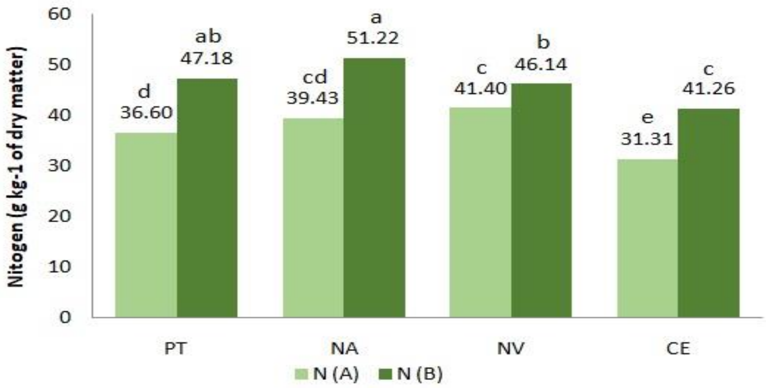


Figure 23. Average amounts of nitrogen (N) (g per kg of dry matter), in *Lolium perenne* cv. 'Benchmark', in group **A** (nitrogen fertilization done once) and group **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE). The light green represents the group **A** and the dark green represents group **B.** The treatments that have the same letter do not differ significantly, according to the LDS test (p = 0.05).

Regarding the amounts of phosphorus (P), there were highly significant differences between treatments but there were no significant differences between groups (p = 0.569) (Figure 24). The plants that grew in the rootzone mixture NA had the higher amounts this macronutrient.

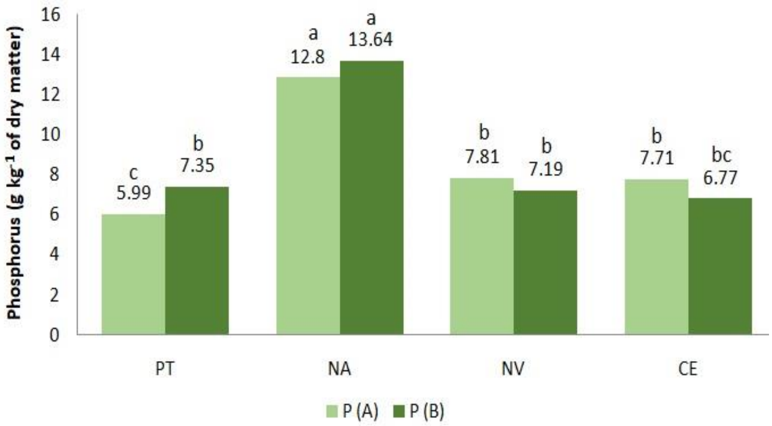


Figure 24. Average amount of total phosphorus (P) in the aerial biomass (g per kg of dry matter), of *Lolium perenne* cv. 'Benchmark', in group **A** (nitrogen fertilization done once) and in group **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE). The light green represents the group **A** and the dark green represents group **B.** The treatments that have the same letter do not differ significantly, according to the LDS test (p = 0.05).

About potassium (K), the interaction between the two groups and the four treatments did not differ significantly (p = 0.725). The amounts of K in the dry matter were higher in the plants from group A and the rootzone mixture that had the higher amount of K was the rootzone mixture NV (Figure 25).

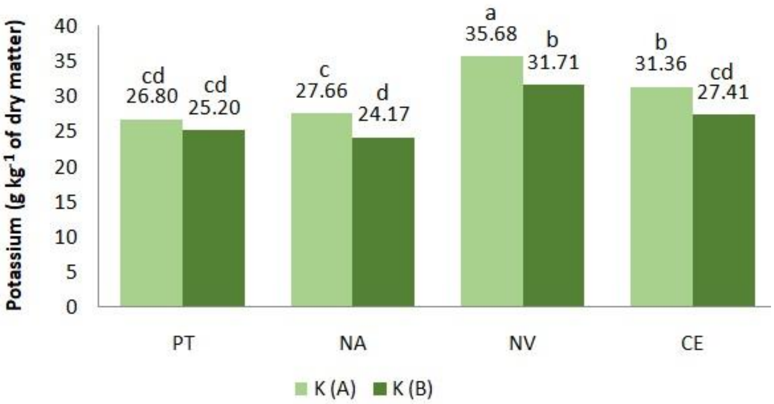


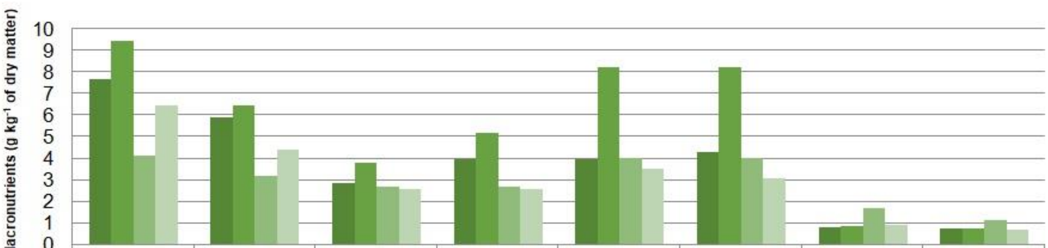
Figure 25. Average amount of total potassium (K) in the aerial biomass of *Lolium perenne* cv. ‘Benchmark’ (g per kg of dry matter), in group **A** (nitrogen fertilization done once) and **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE).The light green represents the group **A** and the dark green represents group **B.** The treatments that have the same letter do not differ significantly, according to the LDS test (p = 0.05).

Regarding the total amount of calcium (Ca) in the plants, the interaction between the groups and treatments was moderately significant (p = 0.036). The amounts of Ca were higher in the plants of group A and the highest values were in the rootzone mixtures NA and PT.

The amounts of magnesium (Mg) were higher in the plants of group B, in the rootzone mixtures NA and PT. In the rootzone mixtures NV and CE, there were no significant differences between groups or between treatments and groups.

The differences of the amounts of sulfur (S) between groups (p = 0.839) and the interaction between the groups and the treatments (p = 0.413), did not differ significantly. The plants with the higher amounts of S were from the rootzone mixture NA.

The amounts of sodium (Na) were higher in the plants of group A and in plants that grew in the rootzone mixture NV (Figure 26).



	Ca (A)	Ca (B)	Mg (A)	Mg (B)	S (A)	S (B)	Na (A)	Na (B)
PT	7.64 **b**	5.89 **c**	2.83 **c**	3.99 **b**	3.94 **bc**	4.30 **b**	0.76 **de**	0.71 **e**
NA	9.44 **a**	6.46 **c**	3.79 **b**	5.17 **a**	8.22 **a**	8.20 **a**	0.85 **cd**	0.71 **e**
NV	4.11 **de**	3.19 **e**	2.68 **c**	2.69 **c**	4.03 **bc**	4.03 **bc**	1.66 **a**	1.13 **b**
CE	6.44 **c**	4.39 **d**	2.58 **c**	2.56 **c**	3.53 **cd**	3.05 **d**	0.89 **c**	0.69 **e**

Figure 26. Average amounts of total calcium (Ca), magnesium (Mg), sulfur (S) and sodium (Na) in the aerial biomassof *Lolium perenne* cv. ‘Benchmark’ (g per kg of dry matter), in the group **A** (nitrogen fertilization done once) and in group **B** (regular fertilization of N every week), in the rootzone mixtures:

peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE). The treatments that have the same letter do not differ significantly, according to the LDS test (p = 0.05).

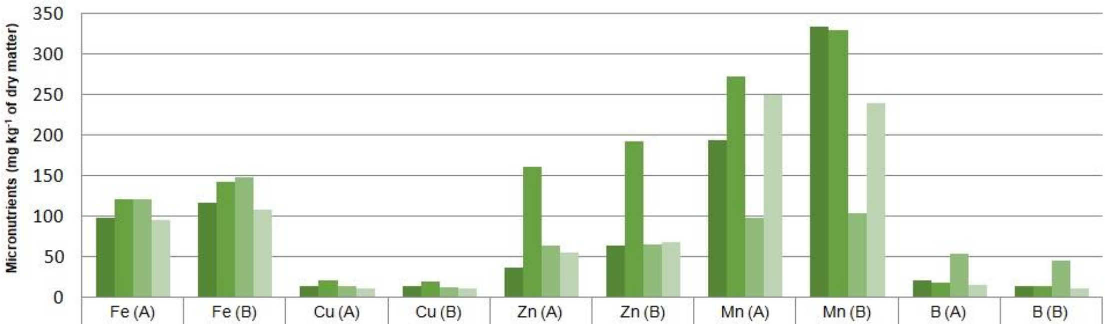
Concerning the micronutrients (Figure 27), the differences of the amounts of iron (Fe), between the two groups and the treatments did not significantly (p = 0.9410). In group A, the rootzone mixtures NA and NV had the higher amounts of Fe. In group B, the amounts were highest in the plants that grew in the rootzone mixture NV, followed by the rootzone mixture NA.

About the amounts of copper (Cu), there were no significant differences between groups and treatments (p = 0.6493). The higher amounts of Cu were found in plants that grew in the rootzone mixture NA, in both groups A and B.

The plants that grew in rootzone mixture NA had the highest amount of zinc (Zn). The amounts were higher in group B, except for rootzone mixtures NV and CE, whose results did not differ significantly between this two treatments and groups. The rootzone mixture PT had the lowest amount of Zn, in group A.

Regarding manganese (Mn), there were highly significant differences between the groups and the treatments (p = 0.003). The amounts were higher in group B, except for NV and CE, whose results had no significant differences between groups and treatments. The rootzone mixtures that had the higher amounts of this micronutrient were PT and NA.

About the amounts of boron (B), the interaction between the groups and the treatments did not differ significantly (p = 0.932). The rootzone mixture NV had the highest amount of B, in both groups.



	Fe (A)	Fe (B)	Cu (A)	Cu (B)	Zn (A)	Zn (B)	Mn (A)	Mn (B)	B (A)	B (B)
PT	96.57 c	116.03 abc	12.19 b	12.26 b	35.36 d	62.89 c	194.15 c	332.88 a	19.76 b	12.33 bc
NA	120.30 abc	141.49 ab	19.96 a	18.20 a	160.13 b	192.07 a	270.74 b	328.57 a	17.56 bc	12.29 bc
NV	119.30 abc	147.83 a	12.43 b	10.75 bc	63.47 c	64.63 c	97.82 d	103.00 d	52.48 a	43.84 a
CE	93.65 c	106.72 bc	9.97 bc	9.55 c	54.73 c	67.14 c	248.79 bc	240.20 bc	14.59 bc	9.43 c

Figure 27. Average micronutrients iron (Fe), copper (Cu), Zinc (Zn), manganese (Mn) and boron (B) contents in the aerial biomassof *Lolium perenne* cv. ‘Benchmark’ (mg per kg of dry matter), in group **A** (nitrogen fertilization done once) and in group **B** (regular fertilization of N every week), in the rootzone mixture: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE). The treatments that have the same letter do not differ significantly, according to the LDS test (p = 0.05).

4.3.2. *Agrostis stolonifera* cv. 'Penn-A4'

Regarding *A. stolonifera*, in the group **A**, due to the characteristics of the rootzone mixture **CE** (cork "earth"), the growth of this grass was poor, which did not allow enough dry matter to be able to analyze correctly the levels of the macronutrients (N, P, K, Ca, Mg, S and the cation Na) and micronutrients (Fe, Cu, Zn, Mn and B) in the plants. The results obtained from the plants that grew in the rootzone mixture CE may have an error associated.

Regarding the amounts of nitrogen (N) in the aerial biomass, there were no significant differences between groups A and B ($p = 0.361$). The plants of group A, from the rootzone mixture CE, had the highest value of N (Figure 28).

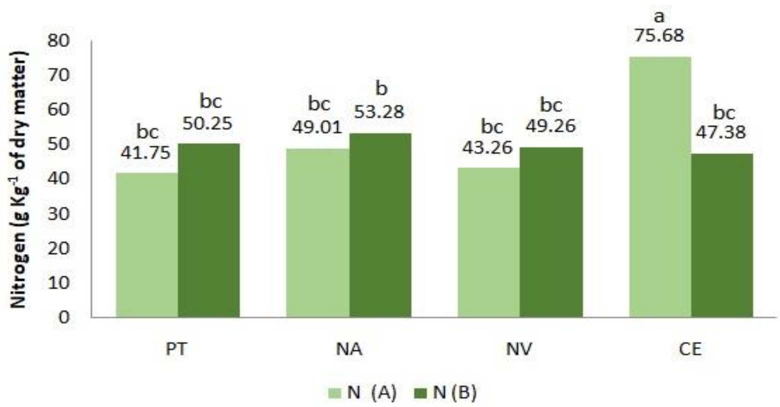


Figure 28. Average amounts of nitrogen (N) (g per kg of dry matter), in *Agrostis stolonifera* cv. 'Penn-A4' in group **A** (nitrogen fertilization done once) and group **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE). The light green represents the group **A** and the dark green represents group **B.** The treatments that have the same letter do not differ significantly, according to the LDS test ($p = 0.05$).

About the amounts of phosphorus (P), with the exception of rootzone mixture CE, in group A, there were no significant differences between groups ($p = 0.569$). The plants that grew in NA rootzone mixture had the higher amounts of P (Figure 29).

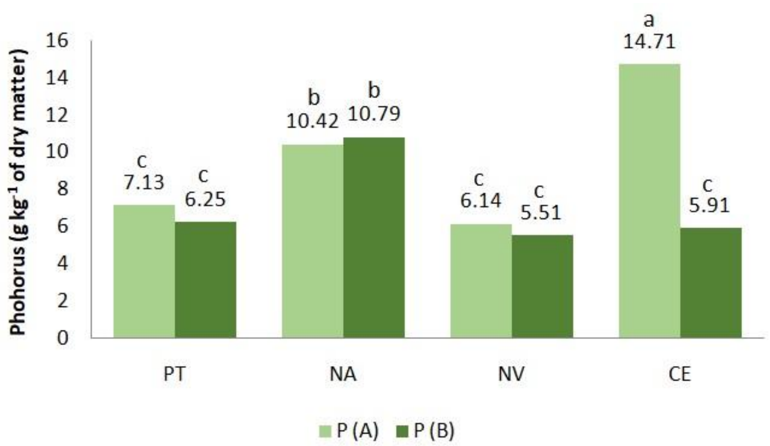


Figure 29. Average amount of total phosphorus (P) in the aerial biomass (g per kg of dry matter), of *Agrostis stolonifera* cv. 'Penn-A4', in group **A** (nitrogen fertilization done once) and in group **B** (regular

fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE). The light green represents the group **A** and the dark green represents group **B**.The treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

Regarding the macronutrient potassium (K), there were no significant differences between treatments and groups, except for the CE rootzone mixture in group A. There is a tendency for the amount of this macronutrient to be bigger in the plants of the group A (Figure 30).

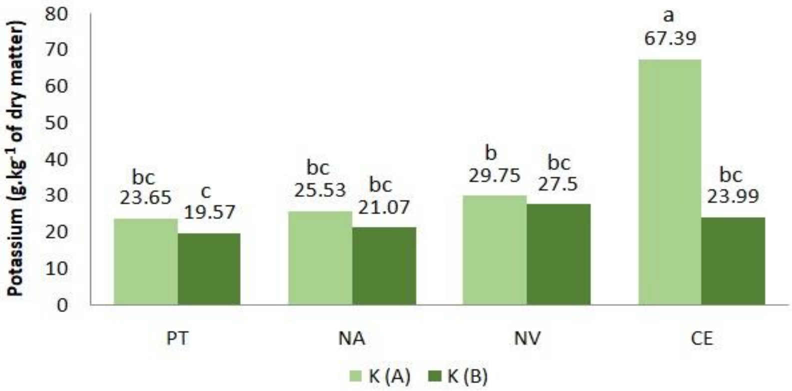


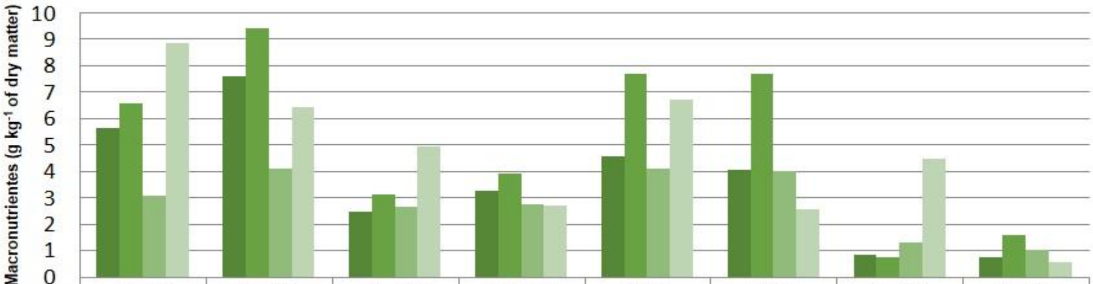
Figure 30. Average amount of total potassium (K) in the aerial biomass of *Agrostis stolonifera* cv. ‘Penn-A4’ (g per kg of dry matter), in group **A** (nitrogen fertilization done once) and **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE). The light green represents the group **A** and the dark green represents group **B**. The treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

Regarding the amounts of the calcium (Ca), there were very significant differences between groups and between treatments. There was an increase of Ca in group B, in all treatments, except for rootzone mixture CE, which presented the highest value in group A and the rootzone mixture NV, whose differences between groups were not significant.

About the amounts of magnesium (Mg) there were no significant differences between groups (p = 0.3757) although they were slightly higher in group B, except for the amount of Mg in the plants of group A, in the rootzone mixture CE, that was much superior.

Regarding the amounts of sulfur (S), there were no significant differences between groups and treatments, with the exception of the rootzone mixture CE that was higher in group A than group B. The plants from the rootzone mixture NA had the higher amount of S. There were no significant differences between the treatment PT and NV, in both groups A and B.

Regarding sodium (Na), there were no significant differences between groups and treatments, except for the rootzone mixture CE, in group A. The rootzone mixtures that had the higher amounts of sodium were the treatment NA, in group B (Figure 31).



	Ca (A)	Ca (B)	Mg (A)	Mg (B)	S (A)	S (B)	Na (A)	Na (B)
PT	5.64 **c**	7.64 **b**	2.46 **d**	3.27 **bc**	4.56 **b**	4.08 **b**	0.86 **bc**	0.74 **bc**
NA	6.60 **bc**	9.44 **a**	3.15 **c**	3.93 **b**	7.73 **a**	7.72 **a**	0.73 **bc**	1.58 **b**
NV	3.08 **d**	4.11 **d**	2.68 **cd**	2.75 **cd**	4.11 **b**	4.01 **b**	1.33 **bc**	1.02 **bc**
CE	8.87 **a**	6.44 **c**	4.95 **a**	2.71 **cd**	6.74 **a**	2.55 **c**	4.47 **a**	0.58 **c**

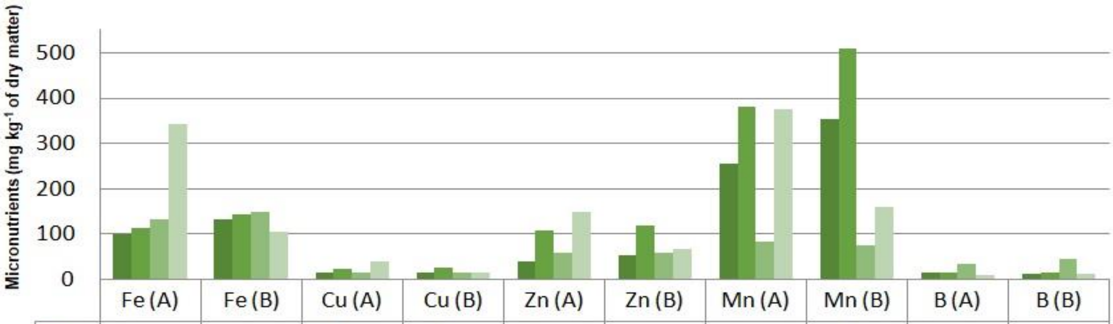
Figure 31. Average amounts of total calcium (Ca), magnesium (Mg), sulfur (S) and sodium (Na) in the aerial biomassof *Agrostis stolonifera* cv. 'Penn-A4' (g per kg of dry matter), in the group **A** (nitrogen fertilization done once) and in group **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork "earth" (CE). The treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

According to Figure 32, regarding the results obtained from the amounts of iron (Fe), there were no significant differences between groups and treatments (p = 0.941) , except for the plants that grew in the rootzone mixture CE, of group A, which presented the highest value of Fe.

Concerning the amounts of copper (Cu), there were no significant differences of the between groups, with the exception of the rootzone mixture CE, in group A. The rootzone mixture that had the highest amount of Cu was the rootzone mixture NA. Similar results were obtained for the micronutrient zinc (Zn).

Regarding manganese (Mn) the average amounts of Mn were higher in the plants of group B, with the exception of the rootzone mixture CE, in group A. There were no significant differences between groups, in the treatment NV. The rootzone mixtures that had the higher amounts of Mn were the NA and PT.

Regarding boron (B), there were highly significant differences between groups and the interaction between groups and the treatments (p = 0.014 and 0.011, respectively). The plants that grew in the rootzone mixture NV had the higher amounts of B, in both groups.



	Fe (A)	Fe (B)	Cu (A)	Cu (B)	Zn (A)	Zn (B)	Mn (A)	Mn (B)	B (A)	B (B)
PT	100.25**b**	130.92**b**	13.34**c**	14.11**c**	39.81 **d**	51.80**cd**	255.21**c**	353.02 **b**	14.81**c**	12.47**cd**
NA	112.55**b**	141.93**b**	24.02**b**	25.72**b**	108.10**b**	119.31**b**	381.00**b**	509.60**a**	13.31**c**	15.13**c**
NV	132.22**b**	147.01**b**	15.11**c**	14.46**c**	58.33**cd**	57.45**cd**	82.76 **e**	75.61 **e**	34.96**b**	43.64**a**
CE	342.35**a**	105.92**b**	40.27**a**	14.60**c**	148.14**a**	67.05 **c**	373.80**b**	159.55**d**	8.12 **d**	11.09**cd**

Figure 32. Average micronutrients iron (Fe), copper (Cu), Zinc (Zn), manganese (Mn) and boron (B) contents in the aerial biomassof *Agrostis stolonifera* cv. ‘Penn-A4’ (mg per kg of dry matter), in group **A** (nitrogen fertilization done once) and in group **B** (regular fertilization of N every week), in the rootzone mixtures: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE). The treatments that have the same letter do not differ significantly, according with the LSD test (p = 0.05).

4.4. Identified weeds

Throughout the development of the experiment, the presence of weeds were surveyed mainly in the pots with rootzone mixture amended with cork “earth” (CE), with the exception of two species that were observed in rootzone mixture with peat (PT) and one in the control NA (sewage sludge and pine bark compost). Regarding the control pots, the emergence of weeds belonging to CE, occurred during the initial phase of the study (May 15th) and the appearance of the weeds in the rootzone mixtures PT and NA occurred in the intermediate phase of the study (beginning of July). The weeds registered and identified during this study are presented in the Table 28.

Table 28. Weeds identified according to the respective rootzone mixture: peat (PT), sewage sludge and pine bark compost (NA), green compost (NV) and cork “earth” (CE).

Rootzone mixture	Clade	Family	Taxone
PT	Eudicotyledoneae	Asteraceae	*Sonchus* sp.
CE	Eudicotyledoneae	Amaranthaceae Asteraceae	*Amaranthus albus* L. *Senecio vulgaris* L.
PT Control	Eudicotyledoneae	Asteraceae	*Ageratina riparia* (Regel) R. M.King& H. Rob.
NA Control	Eudicotyledoneae	Asteraceae	*Sonchus oleraceus* L.

CE Control	Eudicotyledoneae	Solanaceae Asteraceae Caryophyllaceae	*Solanum nigrum* L. *Sonchus oleraceus* L. *Spergula arvensis* L.

The propagation organ of the identified Asteraceae, the pseudofruit cypsela, has attached a structure called pappus (hair-like outgrowths) (Figure 33) that has an important role in the protection of the fruits but also in their dispersal, allowing them to be transported by the wind (Sheldon & Burrows, 1973). Regarding the Amaranthaceae, the Solanaceae and the Caryophyllaceae, the propagation organs are the seeds (Table 29).

Table 29. Geographic distribution, ecology, Raunkiaer life form and the type of fruits produced by the different species identified (adapted from Franco, 1971; 1984; and Scher *et al*., 2015).

Species	Geographic distribution	Ecology	Raunkiaer life form	Flowering season	Fruit	Disper-sion
Sonchus oleraceus	Native of Portugal and of Madeira archipelago. Exotic in Azores archipelago	Present in all the country and it habits in cultivated lands and natural sites	Proto-hemi-cryptophyte	From January to September	Cypsela[1.]	Fruit by wind
Solanum nigrum	Native of Portugal and of Madeira archipelago. Exotic in Azores archipelago	Very common plant, existing in wastelands, ruderalized sites, cultivated land, heaps and in roadsides	Therophyte or Chamaephyte[2.]	Staggered flowering being higher between March and September	Berry	Fruit by birds, rodents and water
Amaranthus albus	Considered exotic in Portugal and nonexistent the Madeira and the Azores archipelago	It is a common plant that is adapted to the different regions of the country	Therophyte	From July to September	Utrile[3.]	Seeds by birds, rodents and water
Senecio vulgaris	Native of Portugal and of Madeira archipelago. Exotic in Azores archipelago	Plant with a high ecological range, present in cultivated lands, meadows and wasteland	Therophyte	From February to April	Cypsela	Fruits by wind

Ageratina riparia	Native in the Madeira archipelago and present in Portugal	Is an invasive plant that prefers mountainous areas with high levels of precipitation	Chamaephyte	From August to October	Cypsela	Fruits by wind and water
Spergula arvensis	Native of Portugal and of Madeira archipelago. Considered exotic in the Azores archipelago	It is a common plant, present in cultivated lands, especially in sandy soils	Therophyte	From February to May	Capsule[4.]	Seeds by birds and mammals, water and agriculture machinery

[1.]False and dry indiscent fruit that contains only one seed (Santo & Monteiro, 2014).
[2.]A perennial woody part with well developed side-branches that appear near the base, no trunk, 30 cm to a 1 m high (Montserrat-Martí *et al.*, 2011).
[3.]Dry dehiscent fruit that only contains one seed (Santo & Monteiro, 2014).
[4.]Dry dehiscent fruit with many seeds (Santo & Monteiro, 2014).

Figure 33. The pappus attached to the pseudo fruit cypsela of *A. riparia* (left) and *S. oleraceus* (right) (adapted from Scher *et al.,* 2015; 2018).

4.5. Fungi identification

There were two fungus species identified during the experiment (Table 30). The fungus that caused an infection and loss of vegetative tissue was the fungus *Bipolaris sorokiniana* (Sacc.) Shoemarker (1959), the anamorph of *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur (1942) (Mycobank, 2016), and it apperead in several pots with different rootzone mixtures. This fungus belongs to the phylum Ascomycota and its conidiophores are characterized by being single or in small groups, septated, smooth, pale or mid dark brown. The conidia is curved, fusoid to broadly ellipsoidal, dark olivaceous brown (Figure 34) (Sivanesan, 1987).

This pathogen agent is the cause of the disease "Leaf Spot/Melting Down", also known as "Blotch Spot", and affects several types of grass including the perennial ryegrass and some varieties of bentgrass. The symptoms are shrinkage and blighted of the leaves and, when the infection is too severe, almost all leaves and tillers die causing severe thinning of the turf mat (Figure 35) (Syngenta, 2011).

The second fungus identified was the macrofungus *Mycena rubromarginata* (Fr.) P. Kumm (1871) (MycoBank, 2016) and this fungus appeared in several pots, but all with the rootzone mixture with the organic amendments cork "earth" (CE). Although it did not infect vegetative tissue, it is innocuous. This fungus belongs to the phylum Basidiomycota and its fruiting structure (carpophores) are characterized by being broad, conical-bell shaped with a striated margin, from brown-purpule to light brown. The gills (or tubes) are white than pink notched (Figure 36). The carpophores are formed at the end of summer - beginning of autumn, in groups, on decomposing organic matter (Intini, 1990). This fungus is a saprophyte that grows on fallen branches and leaves of conifer wood and deciduous trees (Humphrey *et al.*, 2003).

Table 30. The taxonomy of the fungi species identified during the experiment (adapted from NCBI, 2019).

Taxonomy	**Fungi species**	
Phylum	Ascomycota	Basidiomycota
Class	Dothideomycetes	Agaricomycetes
Order	Pleosporales	Agaricales
Family	Pleosporaceae	Mycenaceae
Genus	*Cochliobolus*	*Mycena*
Specie	*C. sativus*	*M. rubromarginata*

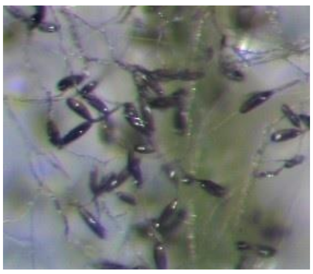


Figure 34. The conidia structures of the fungus *Bipolaris sorokiniana* (Leica®, model MZ 12.5). Image processed by the software LAS V. 4.12.

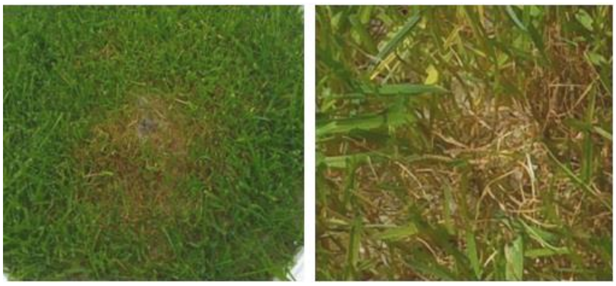


Figure 35. The symptoms of “Leaf Spot” disease: the thinning of the turf mat (left) and the leaf decay (right).

Figure 36. The fruiting structures of *Mycena rubromarginata* growing next to *Agrostis stolonifera* cv. ‘Penn-A4’ (left) and in *Lolium perenne* cv. ‘Benchmark’ (right).

5. Discussion

5.1. Plant development

5.1.1. Aerial biomass production and number of plants

Regarding *L. perenne* cv. 'Benchmark', although the rootzone mixture PT (peat) had the higher amount of biomass produced in the beginning of the experiment, the rootzone mixtures NA (sewage sludge and pine bark compost compost) and NV (green compost) kept increasing the production of biomass, whitout regular nitrogen fertilization, unlike the rootzone mixture PT (Table 8). Comparing the total amount of dry matter produced by the four treatments, NA and NV had the higher amounts of biomass produced and CE (cork "earth") had the lower amount produced (Figure 17).

The results obtained were similar for *A. stolonifera* cv. "Penn-A4" since the rootzone mixture PT also had the higher amount of biomass produced in the shoot harvest V1 and, when the nitrogen fertilization began (V4), the rootzone mixtures NA and NV produced more biomass then the rootzone mixture PT (Table 10).

Comparing the total sum of the biomass produced (group A + B), the rootzone mixture NA had the higher amount of dry matter produced followed by the rootzone mixtures NV and PT (Figure 18). This is due to the fact that composts that have in their composition high nutrient materials like sewage sludge, have more nutrients available than those who are composed by cellulosic plant material, thus allowing the plant growth without regular nitrogen fertilization (Harrison, 2008).

The rootzone mixture CE (cork "earth") had the lowest amount of biomass produced, in both grass species but especially in *A. stolonifera* since it did not produce enough biomass in V1 and V2 to allow a shoot harvest (Table 10).

Regarding the estimated number of plants at the end of the experiment, for both grass species, there were no significant differences between the treatments. Nevertheless, the number of plants obtained in each treatment is lower than the number of seeds sowed in the pots. This is due to the fact that during seed germination and the development of the seedlings (seedling emergence), many factors contribute for the diminution of the number of plants, factors such as sowing conditions (drought and heat), competitiveness between plants by space and nutrients, plagues and diseases (Lamichhan *et al.*, 2018).

5.1.2. Phenological states of the plants

In both grass species, the rootzone mixture CE (cork "earth") showed the least plant phenological evolution and nonexistent tillering. The plants selected from the rootzone mixture PT (peat) had a very similar evolution from the ones selected in the rootzone mixture

NA (sewage sludge and pine bark compost) and NV (green compost) however, the plants from this last two rootzone mixtures presented a higher tillering than the ones from the rootzone mixture PT.

It was determined that the number of plants in each rootzone mixture did not differ significantly but, when the plants were being separated and counted, it was noticeable that the rootzone mixture CE appeared to have a larger number of plants than the other rootzone mixtures, however these plants were less developed and with minor tillering in comparison with the plants provided from the other treatments (annex III). Therefore, the factor that will determine which rootzone mixture is better for the purpose of this experiment is the tillering evolution of the plants, since the tiller density is one of the most important aspects in a golf lawn (Beard, 1973).

It is to take into consideration that, in the same pot, plants with different phenological states are present. This is due to the intrinsic seed genetic diversity and the competition for space and nutrients between them.

5.1.3. Maximum root length and root biomass

In both grass species, the plants that grew in the rootzone mixture NV (green compost) and the rootzone mixture CE (cork “earth”) had the shorter total root length (Table 19). Regarding the result in the rootzone mixture NV, this may be a consequence from the maximum water holding capacity (MWHC) of this rootzone mixture, that is it the highest of all treatments. This means that the plants do not need to increase their root length in order to access water and water accumulation occurred at the bottom of the pots, keeping the oxygen levels low in the rootzone mixture and, consequently, impeding the root from developing in depth.

Regarding the rootzone mixture CE, the shorter total root length presented is not related to its MWHC (since it is the rootzone mixture with the second lowest MWHC) but to the capability of this rootzone mixture to allow the plants to develop.

This would also explain why the plants that grew in the rootzone mixture PT (peat) presented the largest total root length since this rootzone mixture had the lowest MWHC, followed by the rootzone mixture NA (sewage sludge and pine bark compost).

5.2. Chemical and physicochemical characteristics of the rootzone mixtures

For each parameter - (pH, electrical conductivity (EC), exchangeable acidity and organic matter (OM) - the results obtained were similar for both grass species (annexes I and II).

The rootzone mixture NV (green compost) had the higher pH value being classified, according to Soveral-Dias *et al*. (1980), as slightly alkaline. This result goes according to the existing bibliography since the normal pH value of composts, when the materials are stable (at the end of the composting process), is between 7 and 8 (Sempiterno, 2016).

The rootzone mixture NA (sewage sludge and pine bark compost) had the lowest pH value, followed by the rootzone mixture with peat (PT). Both rootzone mixtures are classified as acidic (Soveral-Dias, 1980). Regarding the rootzone mixture PT, the result goes according to the existing bibliography given that peat bogs are known to be very acidic (their pH can vary from 3.6 to 6), due to the decomposition process of the existing vegetative species (Priest, 2012).

However it was the rootzone mixture with sewage sludge and pine bark (NA) that presented the lowest pH value. This may be a result of the characteristics of pine bark as this type of material has a low pH value (Nunes *et al.*, 1999).

Regarding the electrical conductivity (EC) the values in all the treatments are below the minimum acceptable range, except the rootzone mixture NV. The EC value of the rootzone mixture NV (0.56 mS cm^{-1}) is acceptable for most plants but considered high for those species sensitive to salinity (INIAP, 2006).

Concerning the exchangeable acidity, the results are consistent with the results obtained from the pH: the rootzone mixtures that had the lowest pH values – PT and NA – had the highest values of exchangeable acidity and the rootzone mixtures that had the higher pH values – NV and CE (cork "earth") – had the lowest values of exchangeable acidity. For these last two rootzone mixtures, there were no significant differences between them, meaning that these rootzone mixtures do not have acidity.

Regarding the organic matter (OM) by adding peat to the silica sand, the amount of organic matter in the rootzone mixture PT is considered low. Concerning the other three organic amendments, the amount of organic matter in the rootzone mixtures improved but it is considered as medium (Alves, 1989) (annexes I and II). Although the organic amendment cork "earth" (CE) presented the highest content of organic matter (Table 5), when mixed with the silica sand, the differences between the treatments NA and NV did not differ significantly (Tables 20 and 24).

The results of the amounts of the macronutrients phosphorus (P_2O_5) and potassium (K_2O) in the rootzone mixtures were similar for both grass species. The rootzone mixtures that had the higher amounts of phosphorus (P_2O_5) were the rootzone mixtures NA and NV. This result meets the content of P_2O_5 present in the organic amendment sewage sludge and pine bark compost (used in the rootzone mixture NA) which presented the highest amount of P_2O_5, followed by the organic amendment green compost (used in the rootzone mixtures NV) (Table 5).

According to Soveral-Dias *et al*. (1980), the values of P_2O_5 in the rootzone mixtures NA and NV are considered very high (Fertility index 7) (annexes I and II). These high values of P_2O_5 can cause problems of phytotoxicity and have negative consequences on the availability of other nutrients to the plants, *e. g.* antagonism effect with zinc (Varenne, 2006; INIAP, 2006).

Regarding the amount of extractable potassium (K_2O), the rootzone mixture NV (green compost) had the higest amount of this macronutrient followed by the rootzone mixture CE (cork "earth"). This is due to the fact that the organic amendments used in these rootzone mixtures are richer in K_2O than the other organic amendments (Table 5), thus contributing to the increase of this nutrient in the sand-based rootzone mixture.

According to Soveral-Dias *et al.* (1980), the K_2O values obtained, in the species *L. perenne,* in the rootzone mixture NV is considered very high (Fertility index 7) and in the rootzone mixture CE, the value is considered high (Fertility index 4). The value of K_2O obtained, in *A. stolonifera,* in both rootzone mixtures (NV and CE) is also considered very high (Fertility index 7) (annexes I and II). The main problem with high values of K_2O in the soils is the antagonism effect that can exist with other nutrients, namely magnesium (Mg) and calcium (Pii *et al.*, 2015; Jakobsen, 1993).

Regarding the exchangeable bases (Ca, Mg, Na and K), in both grass species, the rootzone mixture that had the lowest amounts of exchangeable bases was the rootzone mixture with peat (PT) followed by the rootzone mixture with sewage sludge and pine bark compost (NA). The rootzone mixture that had the highest amounts of exchangeable bases was the rootzone mixture NV (green compost) (Tables 22 and 26).This result justifies the difference of the pH value of the rootzone mixtures since the saturation degree of the extractable bases is directly associated with the pH value of a soil: the soils with low saturation of bases are soils with low pH value, such as the rootzone mixtures NA and PT (INIAP, 2006).

About the extractable micronutrients (Fe, Cu, Zn and Mn) in the rootzone mixtures, the results were similar for both grass species (annexes I and II). The rootzone mixture PT (peat) had the lowest amount of micronutrients, followed by the rootzone mixture CE (cork "earth"), except for the micronutrient manganese (Mn).

Although cork "earth" is the organic amendment with the highest amount of total Mn (Table 5), in L. perenne this nutrient was the highest in both rootzone mixtures CE and NV (green compost), and there are no significant differences between this two treatments (Table 23). For *A. stolonifera*, the rootzone mixture NV had the highest amount of this micronutrient instead of the rootzone mixture CE (Table 27). This result is not in accordance with the organic amendments composition for the reason that, manganese is an element more available at lower pH values and, in the case of the rootzone mixture NV, the pH value is higher than the rootzone mixture CE (INIAP, 2006).

The rootzone mixture NA (sewage sludge and pine bark compost) had the highest amounts on zinc (Zn) and copper (Cu) (very high and medium, respectively), followed by the rootzone mixture NV (annexes I and II). This result is in agreement with the existing bibliography since the main problem of sewage sludge-based composts (the organic amendment used in the rootzone mixture NA) is their heavy metal content (Ribeiro *et al.*, 2009). Even though the amounts of Zn and Cu are considered high in both rootzone mixtures, the amounts of these elements in the organic amendments are within the maximum values acceptable, according to their class (Table 3).

The rootzone mixture NV had the higher amounts of iron (Fe) followed by the rootzone mixture NA (very high and high, respectively). However, the second organic amendment with the higher value of total Fe is cork “earth” and not sewage sludge and pine bark compost (Table 5). There are many parameters that influence the availability of the nutrients for plants, being pH one of them. Since Naturanat® originated a more acidic rootzone mixture, the Fe is more available for the plants in this mixture (nutrient in its extractable form), than in the rootzone mixture with cork “earth” (CE).

5.3. Mineral composition of the plants

About the amount of nitrogen (N), the plants that grew in the rootzone mixture NA (sewage sludge and pine bark compost) had more N than the ones that grew in the rootzone mixture NV (green compost). The plants from the rootzone mixtures PT (peat) and CE (cork “earth”) had the lower amount of N in the aerial biomass (Figures 23 and 28).

Regarding *Lolium perenne* cv. ‘Benchmark’, the higher amounts of nitrogen (N) were found in the plants of group B (plants that were fertilized with N regurarly). However, the plants in this group produced a lower amount of aerial biomass than the plants from group A (fertilized only once). Since the increase of N fertilization did not augment the amount of biomass produced (Table 9), the plants from group B accumulated this macronutrient in their tissues (luxury consumption) (Costa, 2004).

In *Agrostis stolonifera* cv. ‘Penn-A4’, there were no significant differences between the groups A and B, with the exception of the plants that grew in CE, group A (see 4.3.2.) (Figure 28). This result is due to the fact that, when the plants started being fertilized with N regurarly, the production of biomass increased (Table 11) and consequently the concentration of N in the plants tissues was “diluted” (dilution effect) (Jarrell & Beverly, 1981). For *A. stolonifera*, the presence of N was a limitant factor in the plant growth otherwise the result would similar to the result obtained from *L. perenne.*

The low values of N found in *L. perenne*, in both groups (A and B), in the rootzone mixture CE (cork “earth) suggests that the nature of this organic amendment may have caused a possible immobilization of this macro nutrient (Silva, 2018).

Regarding the amount of phosphorus (P) in *L. perenne*, the plants that grew in the rootzone mixture with sewage sludge and pine bark compost (NA) had the higher amounts of P (12.80 g in group A and 13.64 g in group B) (Figure 24). For *A. stolonifera*, the plants that grew in the rootzone mixture NA also had the higher amounts of P (10.42 g in group A and 10.79 g in group B) and there were no significant differences between treatments (Figure 29), with the exception of the plants that grew in the rootzone mixture CE, in group A (14.71 g) (see 4.3.2.). Since the organic amendment with sewage sludge has a high amount of phosphorus (P_2O_5) in its composition (Table 5) it increased the amount of this nutrient in the respective rootzone mixture (NA) (Fertility index 7). When the amounts of P in the aerial dry matter is bigger then 10 - 20 g, phytotoxicity problems may exist and one of the consequences is a reduced root system (Varennes, 2006). In both grasses the amounts of P are higher than 10 g per kg of dry matter but no phytotoxicity problems were observerd.

About the amount of potassium (K), in *L. perenne*, the plants from the rootzone mixture NV had the higher amounts of K and this macronutrient was slightly higher in plants from group A (Figure 25). This is due to the fact that as the plants keep growing, the amount of potassium in rootzone mixture decreases and that is reflected in the amount of this nutrient in the plants. In *A. stolonifera* there were no significant differences of the amounts of K in the aerial biomass between rootzone mixtures (Figure 30), with the exception of the plants that grew in the rootzone mixture CE, in group A (see 4.3.2.). The results obtained from *A. stolonifera* were different than those obtained from *L. perenne* because different plants have different nutrients needs.

About the secondary macronutrients (Ca, Mg and S) and the cation Na, in both grass species, the plants that grew in the rootzone mixture NA (sewage sludge and pine bark compost) and the rootzone mixture PT (peat) had the higher amounts of calcium (Ca) and magnesium (Mg). However the rootzone mixtures that had more Ca and Mg available for the plants were the rootzone mixtures NV (green compost) and CE (cork “earth”) (Tables 22 and 26). In *L. perenne*, the plants that grew in these last two rootzone mixtures had the lowest amounts of Ca and Mg (Figure 26). Regarding *A. stolonifera*, the results are very similar (Figure 31), with the exception of the plants that grew in the rootzone mixture CE, in group A (see 4.3.2.).

This result suggests that there could have been an antagonism effect between the potassium (K_2O) present in the rootzone mixtures NV and CE and the availability of calcium (Ca) and magnesium (Mg) for the plants. Calcium and magnesium themselves are antagonists which means that the excess of one element prevents the other from being absorved (Jakobsen, 1993). However, in both grass species and in all rootzone mixtures, the relation exchangeable Ca:Mg is considered high (more calcium than magnesium) which means that nutricional imbalances can occur (annexes I and II).

In both grass species, there were no significant differences of the amounts of sulfur (S) in the dry matter between groups and treatments, with the exception of the plant species *A. stolonifera* that grew in the rootzone mixture CE, in group A (see 4.3.2.) and except for the plants that grew in the rootzone mixture NA (Figures 26 and 31). Even though the plants that grew in the rootzone mixture NA had the highest amount of S, both organic amendments sewage sludge and pine bark compost (used in the rootzone mixture NA) and the green compost (used in the rootzone mixture NV) are equally high in sulfur (Table 5).

The difference of the amount of sulfur in the plants that grew in the rootzone mixture NA and NV may be due to the fact that the organic amendment NA (rich in sewage sludge) tends to mineralize more than the organic amendment NV, thus liberating more sulfur. The composts usually are more stable and present slow mineralization, making available small amounts of nutrients, such as sulfur (Varennes, 2006). This result meets those obtained from the amount of nitrogen in the plants. Like nitrogen, sulfur is an organic compound and the plants that grew in the rootzone mixture NA had higher values of N and S.

Regarding the amount of sodium (Na) in the aerial dry matter, in *L. perenne,* the plants that grew in the rootzone mixture NV (green compost) had the higher amounts of Na (Figure 26). In *A. stolonifera*, with the exception of the plants that grew in the rootzone mixture CE in group A (see 4.3.2.), there were no significant differences of the amounts of this nutrient between the treatments, although is slightly higher in the plants that grew in the rootzone mixture NA in group B (Figure 31). This result, with the exception of the rootzone mixture CE, goes according to the existing bibliography since one of the problems associated with the use of compost is the salt content (Ribeiro *et al*., 2000).

Concerning the micronutrients (Fe, Cu, Zn, Mn and B) content in the aerial dry matter of the plants, with the exception for the plants of *A. stolonifera* cv. 'Penn-A4' that grew in the rootzone mixture CE in group A (see 4.3.2.), the results obtained in both grass species were similar in both grass species (Figure 27 and 32).

About amount of iron (Fe) in the plants, except for *A. stolonifera* (rootzone mixture CE in group A) there were no significant differences between treatments although in *L. perenne,* they were slightly higher in the plants that grew in the rootzone mixture NV (green compost) in group B. This result is due to the fact that the rootzone mixture amended with green compost had the higher amount of exctratable Fe available for the plants.

The amount of copper (Cu) was higher in the plants that grew in the rootzone mixture NA (sewage sludge and pine bark compost), except for the plants of *A. stolonifera* that grew in the rootzone mixture CE, in group A (see 4.3.2.). This result meets the result obtained from the rootzone mixture amended with sewage sludge and pine bark compost (Tables 23 and 27) since this organic amendment has the higher amount of Cu (Table 5).

The plants that had the higher amount of zinc (Zn) were the ones that grew in the rootzone mixture NA and the differences of the amounts between the other treatments did not differ significantly, with the exception of *A. stolonifera* that grew in the rootzone mixture CE, in group A. This result is justified with the amount of extractable Zn in the rootzone mixture amended with sewage sludge and pine bark compost, which is considered very high for both grass species (annexes I and II). When the amount of phosphorus (P_2O_5) in the soils is high it can cause an antagonist effect in the element Zn (INIAP, 2006). However since the rootzone mixture NA has also very high amounts of Zn, this antagonism between nutrients is overcome.

Regarding the amount of manganese (Mn) in the aerial biomass, the plants that grew in the rootzone mixtures PT, NA and CE had the higher amounts of this micronutrient. However the rootzone mixtures that had higher amounts of exctractable Mn were the rootzone mixtures NV and CE (Tables 23 and 27). There are many factors that can influence the availability of the nutrients for the plants, *e. g.* the pH and since the the availability of Mn is higher in low pH value, the plants that grew in the rootzone mixtures PT e NA, had this micronutrient more available, for the reason that these rootzone mixtures are classified as acidic and very acidic, respectively (Soveral-Dias *et al*., 1980).

The result obtained for boron (B) in the aerial biomass in both grass species, meets the amount of this micronutrient in the rootzone mixtures: the plants that grew in the rootzone mixture NV had the higher amounts of B since the organic amendment used (green compost) is also the one with the highest amount of B (Table 5).

5.4. Weed identification

As might be expected, it was found that most of the weeds emerged in the rootzone mixture CE (cork "earth"). This is due to the fact that this organic amendment is composed by a considerable amount of soil that, it self had a seed-bank (Table 28). The different *taxa* identified in this rootzone mixture are explained by the fact that the cork "earth" product is collected in different parts of the country, *i. e.* different habitats have different plants thriving in them.

Regarding the pots control PT (peat) and control NA (sewage sludge and pine bark compost), the weeds identified (*S. oleraceus* and *A. riparia*) (Table 29) could have been result of local contamination for the reason that there was no register of their appearance in other pots, with the same respective rootzone mixture, and both of this species were naturally present in the site where the study took place. It is because of the nature of the pseudo fruits of these species that permitted their presence where they should not exist (Sheldon & Burrows, 1973).

5.5. Fungi identification

The fungus *Bipolaris sorokiniana* is a pathogen that remains active in infected crop residues, secondary hosts, as dormant conidia in the soils and it is seedborne (Acharya *et al.*, 2011). The conidiophores can be transmitted by rain and wind but the infected seeds are the primarily source of inoculums and the main source of leaf blight (Shane, 1981).

Since the infected plants were present in different types of rootzone mixtures and protected from the rain, it is unlikely that the pathogen was present in the rootzone mixture or transmitted by rain. The infection occurred either from infected seeds or by conidiophores that were transported by the wind, from secondary hosts near the experimental place.

The fruiting structures of the fungus *Mycena rubromarginata* appeared in several pots but only in those with the rootzone mixture with the organic amendment, cork "earth" (CE). Although it is associated with conifer fallen branches and leaves from deciduous trees, due to the nature of this residue and being this fungus is a saprophyte, it is possible that this organic amendment presented the right characteristics for the fungal growth, since it is composed by dead organic matter. Associated with *Quercus* spp. is also the species *M. haematopus* and *M. galericulata* (Intini, 1990).

The presence of this type of fungus (Basidiomycota) (Table 30) is problematic to golf courses because they cause irregularities on the turfgrass, therefore compromising the playability of the course and to treat it implies the use of more fungicides or even replace the rootzone mixture (Bardgett, 2016).

6. Conclusions and future investigations

The results obtained show that the production of aerial biomass of both grass species *Agrostis stolonifera* cv. 'Penn-A4' and *Lolium perenne* cv. 'Benchmark' were higher in the rootzone mixture amended with sewage sludge and pine bark compost (NA) followed by the rootzone mixture amended with green compost (NV), in comparison with the rootzone mixture amended with peat (PT). The rootzone mixture that showed the least amount of aerial biomass produced, for both grass species, and the least plant development was the rootzone mixture amended with cork "earth" (CE).

By comparing the plant development and tillering, the development of the plants *L. perenne* cv. 'Benchmark' was very similar between the four rootzone mixtures, with the exception of the the plants that grew in the rootzone mixture CE that presented minor tillering. Regarding the grass *A. stolonifera* cv. 'Penn-A4', the plants that grew in the rootzone mixture PT showed higher development and tillering until the middle of the experiment (July 9^{th}). At the end of the experiment (July 25^{th}), the plants that grew in the rootzone mixture NA presented higher development and tillering.

The results obtained from the root biomass produced were very similar for both grass species. The plants that grew in the rootzone mixture PT had the highest amount of root biomass followed by the other three rootzone mixtures, whose differences did not differ significantly. However, the total root length in the respective rootzone mixtures were significantly different, which suggests that the nature of organic amendments used have an influence in the capability of the root to develop in depth but also influence the lateral root development, *e. g.* the root biomass produced in the rootzone mixtures NA and NV did not differ significantly but the total root length in the rootzone mixture NV was significantly lower than the rootzone mixture NA.

By adding these organic amendments (NA, NV and CE) in the sand-base rootzone mixture, the amounts of nutrients available for the plants growth increased considerably by comparison with the peat. The rootzone mixture that came closest to the rootzone mixture PT was the rootzone mixture NA, with the exception of the amounts of extractable phosphorus (P_2O_5), iron (Fe), copper (Cu) and zinc (Zn), whose amounts were higher.

Despite the low pH value of the rootzone mixtures PT and NA the biomass production was higher in these rootzone mixtures, meaning that the low pH was not a limiting factor for the plants development (nutrients availability).

The four- and three - week nitrogen fertilization gaps, between *L. perenne* and *A. stolonifera* shoot harvests, allowed to evaluate the residual nitrogen effect of the organic amendments, in the plants growth. For both grass species, the rootzone mixture with sewage sludge and pine bark compost (NA) allowed the increase of the plants development without regular nitrogen fertilization, followed by the rootzone mixture amended with green compost (NV). This experiment also allowed observing that the nitrogen was a limiting factor for the *A. stolonifera* growth and not for the grass *L. perenne*, which in turn accumulated N in its leaves.

The rootzone mixtures NA and NV had the higher amounts of extractable phosphorus (P_2O_5) (Fertility index 7 for both rootzone mixtures) and althought these values of extractable P_2O_5 were very high, no problems with phytotoxicity were observed during this experiment, and the possible antagonism effect between P_2O_5 and zinc (Zn) was overcome with the high amount of this element present in the organic amendement sewage sludge and pine bark compost.

The rootzone mixtures NV and CE had the higher amounts of extractable potassium (K_2O) (Fertility index 7 and 4, respectivly) and the higher amounts of exchangeable calcium (Ca) and (Mg). The plant analysis showed that the plants that grew in these rootzone mixtures had the higher amounts of total K but consequently the lower amount of total Ca and Mg which suggests that there was a possible antagonism effect between the elements K, Ca and Mg. However during this experiment, the plants did not show any signs of nutrient deficiency.

Regarding the weeds identified during this experiment, most are dicotyledons that belong to the therophyte and chamaephyte Raunkiaer life form and the key structure to control this type of weeds is their hypocotyls. When this structure is severely damaged or cut completely, the two systems that a plant need to live (water and nutrient collection system from the roots and the photosynthesis system performed in the leaves) are separated and the plant will die (Merfield, 2019).

In golf courses the turf is mowed every day, at a low mowing height because it provides playability benefits. The putting green is the area whose cut is the lowest and it can be as low as 3.17 millimeters, depending on the species used (USGA, 2018). At low mowing heights the weeds present, with the exception of *Sonchus oleraceus* (proto-hemicryptophyte), the chamaephytes would not resist such low mowing cuts since their renewal meristems are close to the ground (within 25 - 50 centimeters above ground) and the therophytes, that are annual plants that die after seed production and with the daily mowing, would be damaged before producing seeds (Mueller-Dombois & Ellenberge, 1966).

The only weed that could be problematic is the Asteraceace *S. oleraceus.* This species would resist to low mowing cut since the renewal meristems are located very close to the ground (protected by ground debris), thus being necessary to remove it by manually or with herbicide application.

The infection of the fungus *Bipolaris sorokiniana* in *A. stolonifera* occurred either from infected plants (secondary hosts) present nearby the nursery where the experiment took place or the pathogen was already present in infected seeds. The presence of *Mycena rubromarginata* in the rootzone mixtures amended with cork "earth" (CE), occurred due to the nature of the organic amendment since this fungus is associated with decomposing organic matter.

By comparing the results obtained from the rootzone mixtures amended with the different organic amendments (the aerial biomass production, tillering and plant development, along with the increase of nutrients in the sand-base rootzone mixture), with the costs of the organic amendments, per hectare, with a ratio of 80% : 20%, the best cost benefit ratio is obtained from the organic amendment composed by sewage sludge and pine bark (NA) (approximately 5 times less expensive that the peat used in this experiment). Althought the residue cork "earth" does not have any costs, the results obtained from this cork residue were not the most promessing. The second best cost benefit ratio comes with the selection of the green compost (NV) as an organic amendment (approximately 4.5 times less expensive than the peat used in this experiment).

With the results obtained, in the field, further studies are needed to evaluate the performance of these organic amendments, as sand-based rootzone mixtures for putting greens, to determine the biomass production, plant development and tillering of the grass species used (and other species) as well as the physical, chemical and biological changes that could occur in the rootzone mixture and its profile.

With the amounts of nutrients that are naturally present in the organic amendments, there could be an opportunity to study the effect of different amounts of fertilizer on the biomass production or even test different mixtures between different organic amendments, in order to minimize the amounts of fertilizers inputs.

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Annex I

The organic matter (OC), pH and electrical conductivity (EC), the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the Cation Exchange Capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents of the four rootzone mixtures: peat (PT), sewage sludge with pine bark compost (NA), green compost (NV) and cork “earth” (CE), where *Lolium perenne* cv. ‘Benchmark’ grew.

Table 1. The organic (OC), pH and electrical conductivity (EC), the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cationic exchange capacity (CEC), relation between exchangeable Ca:Mg, and exchangeable K:Mg and the extractable micronutrients (Fe, Cu, Zn, Mn) contents in the rootzone mixtures: peat (PT), sewage sludge with pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Parameters	**Units of measure**	**Peat** (PT)	**Sewage sludge and pine bark compost** (NA)	**Green compost** (NV)	**Cork "earth"** (CE)
Organic matter (OM)	%	0.91 **c**	2.27 **ab**	2.94 **a**	1.61 **bc**
pH (H_2O) (1:2.5)		4.90 **c**	4.33 **d**	8.78 **a**	7.10 **b**
Electrical conductivity (EC) (1:2)	mS cm^{-1}	0.09 **c**	0.24 **b**	0.56 **a**	0.15 **bc**
Extractable phosphorus (P_2O_5)	mg kg^{-1}	21.49 **b**	356.19 **a**	268.43 **a**	37.22 **b**
Extractable potassium (K_2O)	mg kg^{-1}	6.12 **c**	14.46 **c**	414.34 **a**	106.04 **b**
Calcium (exchangeable base) (Ca)	cmol(+) kg^{-1}	0.59 **c**	1.90 **b**	9.95 **a**	1.49 **bc**
Magnesium (extractable base) (Mg)	cmol(+) kg^{-1}	0.14 **b**	0.31 **b**	1.43 **a**	0.24 **b**
Sodium (extractable base) (Na)	cmol(+) kg^{-1}	0.008 **b**	0.02 **b**	0.31 **a**	0.02 **b**
Potassium (extractable base) (K)	cmol(+) kg^{-1}	0.01 **c**	0.03 **c**	0.88 **a**	0.23 **b**
Cationic Exchange Capacity (CEC)	cmol(+) kg^{-1}	0.95	2.67	12.68	2.07
Relation exchangeable Ca / exchangeable Mg		4.21	6.12	6.95	6.20
Relation exchangeable K / exchangeable Mg		0.07	0.10	0.62	0.95
Extractable iron (Fe)	mg kg^{-1}	9.39 **c**	45.61 **b**	101.32 **a**	17.02 **c**
Extractable copper (Cu)	mg kg^{-1}	0.26 **c**	2.91 **a**	1.07 **b**	0.31 **c**
Extractable zinc (Zn)	mg kg^{-1}	1.15 **b**	25.22 **a**	3.19 **b**	0.76 **b**
Extractable manganese (Mn)	mg kg^{-1}	1.54 **c**	5.08 **b**	8.85 **a**	9.04 **a**

In each row, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).

Table 2. The classification of the rootzone mixture with peat (PT) according to the organic matter (OM) content, the pH and electrical conductivity (EC) value, the extractable phosphorus (P_2O_5) and extractable potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	Units of measure	Peat (PT)	Observations
Organic matter (OM)[a]	%	0.91	Low
pH (H_2O) (1:2.5)[b]		4.90	Acidic
Electrical conductivity (EC) (1:2)[c]	mS cm^{-1}	0.09	Soil without salinity effects
Extractable phosphorus (P_2O_5)[b]	mg kg^{-1}	21.49	Very low (Fertility index 1)
Extractable potassium (K_2O)[b]	mg kg^{-1}	6.12	Very low (Fertility index 1)
Calcium (exchangeable base) (Ca)[a]	cmol(+) kg^{-1}	0.59	Very low
Magnesium (extractable base) (Mg)[a]	cmol(+) kg^{-1}	0.14	Very low
Sodium (extractable base) (Na) [a]	cmol(+) kg^{-1}	0.008	Very low
Potassium (extractable base) (K)[a]	cmol(+) kg^{-1}	0.01	Very low
Cationic Exchange Capacity (CEC)[a]	cmol(+) kg^{-1}	0.95	Very low
Relation exchangeable Ca / exchangeable Mg[d]		4.21	High Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg[e]		0.07	Low Unfavorable for plant nutrition in K
Extractable iron (Fe)[f]	mg kg^{-1}	9.39	Very low
Extractable copper (Cu)[f]	mg kg^{-1}	0.26	Very low
Extractable zinc (Zn)[f]	mg kg^{-1}	1.15	Low
Extractable manganese (Mn)[f]	mg kg^{-1}	1.54	Very low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Table 3. The classification of the rootzone mixture with sewage sludge and pine bark compost (NA) according to the organic matter (OM) content, the pH and electrical conductivity (EC) values, the extractable phosphorus (P_2O_5) and extractable potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	Units of measure	Sewage sludge and pine bark compost (NA)	Observations
Organic matter (OM)[a]	%	2.27	Medium
pH (H_2O) (1:2.5)[b]		4.33	Very acidic
Electrical conductivity (EC) (1:2)[c]	mS cm^{-1}	0.24	Soil without salinity effects
Extractable phosphorus (P_2O_5)[b]	mg kg^{-1}	356.19	Very high (Fertility index 7)
Extractable potassium (K_2O)[b]	mg kg^{-1}	14.46	Very low (Fertility Index 1)
Calcium (exchangeable base) (Ca)[a]	cmol(+) kg^{-1}	1.90	Very low
Magnesium (extractable base) (Mg)[a]	cmol(+) kg^{-1}	0.31	Very low
Sodium (extractable base) (Na) [a]	cmol(+) kg^{-1}	0.02	Very low
Potassium (extractable base) (K)[a]	cmol(+) kg^{-1}	0.03	Very low
Cationic Exchange Capacity (CEC)[a]	cmol(+) kg^{-1}	2.67	Very low
Relation exchangeable Ca / exchangeable Mg[d]		6.12	High Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		0.10	Low Unfavorable for plant nutrition in K
Extractable iron (Fe)[f]	mg kg^{-1}	45.61	High
Extractable copper (Cu)[f]	mg kg^{-1}	2.91	Medium
Extractable zinc (Zn)[f]	mg kg^{-1}	25.22	Very high
Extractable manganese (Mn)[f]	mg kg^{-1}	5.08	Very low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Table 4. The classification of the rootzone mixture with green compost (NV) according to the organic matter (OM) content, the pH and electrical conductivity (EC) values, the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	**Units of measure**	**Green compost** (NV)	**Observations**
Organic matter (OM)[(a)]	%	2.94	Medium
pH (H_2O) (1:2.5)[(b)]		8.78	Alkaline
Electrical conductivity (EC) (1:2)[(c)]	mS cm^{-1}	0.56	Soil little saline
Extractable phosphorus (P_2O_5)[(b)]	mg kg^{-1}	268.43	Very high (Fertility index 7)
Extractable potassium (K_2O)[(b)]	mg kg^{-1}	414.34	Very high (Fertility index 7)
Calcium (exchangeable base) (Ca)[(a)]	cmol(+) kg^{-1}	9.95	Medium
Magnesium (extractable base) (Mg)[(a)]	cmol(+) kg^{-1}	1.43	Medium
Sodium (extractable base) (Na) [(a)]	cmol(+) kg^{-1}	0.31	Medium
Potassium (extractable base) (K)[(a)]	cmol(+) kg^{-1}	0.88	High
Cationic Exchange Capacity (CEC)[(a)]	cmol(+) kg^{-1}	12.68	Medium
Relation exchangeable Ca / exchangeable Mg[(d)]		6.95	High Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		0.62	High Unfavorable for plant nutrition in Mg
Extractable iron (Fe)[(f)]	mg kg^{-1}	101.32	Vey high
Extractable copper (Cu)[(f)]	mg kg^{-1}	1.07	Medium
Extractable zinc (Zn)[(f)]	mg kg^{-1}	3.19	Medium
Extractable manganese (Mn)[(f)]	mg kg^{-1}	8.85	Low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Table 5. The classification of the rootzone mixture with cork “earth” (CE) according to the pH and electrical conductivity (EC) values, the extractable phosphorus (P_2O_5) and extractable potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg, and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	**Units of measure**	**Cork “earth”** (CE)	**Observations**
Organic matter (OM)[a]	%	1.61	Medium
pH (H_2O) (1:2.5)[b]		7.10	Neutral
Electrical conductivity (EC) (1:2)[c]	mS cm^{-1}	0.15	Soil without saline effects
Extractable phosphorus (P_2O_5)[b]	mg kg^{-1}	37.22	Low (Fertility index 2)
Extractable potassium (K_2O)[b]	mg kg^{-1}	106.04	High (Fertility index 6)
Calcium (exchangeable base) (Ca)[a]	cmol(+) kg^{-1}	1.49	Very low
Magnesium (extractable base) (Mg)[a]	cmol(+) kg^{-1}	0.24	Very low
Sodium (extractable base) (Na) [a]	cmol(+) kg^{-1}	0.02	Very low
Potassium (extractable base) (K)[a]	cmol(+) kg^{-1}	0.23	Low
Cationic Exchange Capacity (CEC)[a]	cmol(+) kg^{-1}	2.07	Very low
Relation exchangeable Ca / exchangeable Mg[d]		6.20	High Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		0.95	High Unfavorable for plant nutrition in Mg
Extractable iron (Fe)[f]	mg kg^{-1}	17.02	Low
Extractable copper (Cu)[f]	mg kg^{-1}	0.31	Very Low
Extractable zinc (Zn)[f]	mg kg^{-1}	0.76	Low
Extractable manganese (Mn)[f]	mg kg^{-1}	9.04	Low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Annex II

The organic matter (OC), pH and electrical conductivity (EC), the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the Cation Exchange Capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents of the four rootzone mixtures: peat (PT), sewage sludge with pine bark compost (NA), green compost (NV) and cork “earth” (CE), where *Agrostis stolonifera* cv. ‘Penn-A4’ grew.

Table 1. The organic matter (OC), pH, electrical conductivity (EC), the cationic exchange capacity (CEC), the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cationic exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents in the four rootzone mixtures: peat (PT), sewage sludge with pine bark compost (NA), green compost (NV) and cork "earth" (CE).

Parameters	**Units of measure**	**Peat** (PT)	**Sewage sludge and pine bark compost** (NA)	**Green compost** (NV)	**Cork "earth"** (CE)
Organic matter (OM)	%	0.93 **b**	2.26 **a**	2.76 **a**	2.01 **a**
pH (H_2O) (1:2.5)		4.36 **c**	4.25 **d**	8.50 **a**	6.42 **b**
Electrical conductivity (EC) (1:2)	mS cm^{-1}	0.08 **b**	0.20 **b**	0.56 **a**	0.13 **b**
Extractable phosphorus (P_2O_5)	mg kg^{-1}	23.89 **b**	259.45 **a**	286.52 **a**	46.19 **b**
Extractable potassium (K_2O)	mg kg^{-1}	9.26 **c**	12.63 **c**	452.97 **a**	222.57 **b**
Calcium (exchangeable base) (Ca)	cmol(+) kg^{-1}	0.87 **b**	1.25 **b**	12.19 **a**	1.99 **b**
Magnesium (extractable base) (Mg)	cmol(+) kg^{-1}	0.17 **b**	0.18 **b**	1.66 **a**	0.27 **b**
Sodium (extractable base) (Na)	cmol(+) kg^{-1}	0.02 **b**	0.02 **b**	0.34 **a**	0.01 **b**
Potassium (extractable base) (K)	cmol(+) kg^{-1}	0.02 **c**	0.03 **c**	0.96 **a**	0.47 **b**
Cationic Exchange Capacity (CEC)	cmol(+) kg^{-1}	1.29	1.86	15.24	2.82
Relation exchangeable Ca / exchangeable Mg		5.12	6.94	7.34	7.37
Relation exchangeable K / exchangeable Mg		0.12	0.17	0.58	1.74
Extractable iron (Fe)	mg kg^{-1}	9.80 **c**	42.65 **ab**	58.51 **a**	22.59 **bc**
Extractable copper (Cu)	mg kg^{-1}	0.20 **c**	2.43 **a**	1.31 **b**	0.35 **c**
Extractable zinc (Zn)	mg kg^{-1}	0.53 **c**	22.16 **a**	4.29 **b**	1.29 **bc**
Extractable manganese (Mn)	mg kg^{-1}	4.36 **d**	6.68 **c**	14.06 **a**	12.01 **b**

In each row, the treatments that have the same letter do not differ significantly, according with the LSD test ($p = 0.05$).

Table 2. The classification of the rootzone mixture with peat (T) according to the organic matter (OM) content, the pH and electrical conductivity (EC) value, the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameter	Unit	Peat (PT)	Observations
Organic matter (OM)[a]	%	0.93	Low
pH (H_2O) (1:2.5)[b]		4.36	Very acidic
Electrical conductivity (EC) (1:2)[c]	mS cm^{-1}	0.08	Soil without saline effects
Extractable phosphorus (P_2O_5)[b]	mg kg^{-1}	23.89	Low (Fertility index 2)
Extractable potassium (K_2O)[b]	mg kg^{-1}	9.26	Very low (Fertility index 1)
Calcium (exchangeable base) (Ca)[a]	cmol(+) kg^{-1}	0.87	Very low
Magnesium (extractable base) (Mg)[a]	cmol(+) kg^{-1}	0.17	Very low
Sodium (extractable base) (Na) [a]	cmol(+) kg^{-1}	0.02	Very low
Potassium (extractable base) (K)[a]	cmol(+) kg^{-1}	0.02	Very low
Cationic Exchange Capacity (CEC)[a]	cmol(+) kg^{-1}	1.29	Very low
Relation exchangeable Ca / exchangeable Mg[d]		5.12	High. Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		0.12	Low Unfavorable for plant nutrition in K
Extractable iron (Fe)[f]	mg kg^{-1}	9.80	Very low
Extractable copper (Cu)[f]	mg kg^{-1}	0.20	Very low
Extractable zinc (Zn)[f]	mg kg^{-1}	0.53	Very low
Extractable manganese (Mn)[f]	mg kg^{-1}	4.36	Very low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Table 3. The classification of the rootzone mixture with sewage sludge and pine bark compost (NA) according to the organic matter (OM) content, the pH and electrical conductivity (EC) value, the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	**Units of measure**	**Sewage sludge and pine bark compost** (NA)	**Observations**
Organic matter (OM)[(a)]	%	2.26	Medium
pH (H_2O) (1:2.5)[(b)]		4.25	Very acidic
Electrical conductivity (EC) (1:2)[(c)]	mS cm^{-1}	0.20	Soil without saline effects
Extractable phosphorus (P_2O_5)[(b)]	mg kg^{-1}	259.45	Very high (Fertility index 7)
Extractable potassium (K_2O)[(b)]	mg kg^{-1}	12.63	Very low (Fertility index 1)
Calcium (exchangeable base) (Ca)[(a)]	cmol(+) kg^{-1}	1.25	Very low
Magnesium (extractable base) (Mg)[(a)]	cmol(+) kg^{-1}	0.18	Very low
Sodium (extractable base) (Na) [(a)]	cmol(+) kg^{-1}	0.02	Very low
Potassium (extractable base) (K)[(a)]	cmol(+) kg^{-1}	0.03	Very low
Cationic Exchange Capacity (CEC)[(a)]	cmol(+) kg^{-1}	1.86	Very low
Relation exchangeable Ca / exchangeable Mg[(d)]		6.94	High. Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		0.17	Low Unfavorable for plant nutrition in K
Extractable iron (Fe)[(f)]	mg kg^{-1}	42.65	High
Extractable copper (Cu)[(f)]	mg kg^{-1}	2.43	Medium
Extractable zinc (Zn)[(f)]	mg kg^{-1}	22.16	Very high
Extractable manganese (Mn)[(f)]	mg kg^{-1}	6.68	Very low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Table 4. The classification of the rootzone mixture with green compost (NV) according to the organic matter (OM) content, the pH and electrical conductivity (EC) value, the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg and exchangeable K:/Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	Units of measure	Green compost (NV)	Observations
Organic matter (OM)[(a)]	%	2.76	Medium
pH (H_2O) (1:2.5)[(b)]		8.50	Little alkaline
Electrical conductivity (EC) (1:2)[(c)]	mS cm^{-1}	0.56	Very little saline soil
Extractable phosphorus (P_2O_5)[(b)]	mg kg^{-1}	286.52	Very high (Fertility index 7)
Extractable potassium (K_2O)[(b)]	mg kg^{-1}	452.97	Very high (fertility Index 7)
Calcium (exchangeable base) (Ca)[(a)]	cmol(+) kg^{-1}	12.19	High
Magnesium (extractable base) (Mg)[(a)]	cmol(+) kg^{-1}	1.66	Medium
Sodium (extractable base) (Na) [(a)]	cmol(+) kg^{-1}	0.34	Medium
Potassium (extractable base) (K)[(a)]	cmol(+) kg^{-1}	0.96	Very low
Cationic Exchange Capacity (CEC)[(a)]	cmol(+) kg^{-1}	15.24	Medium
Relation exchangeable Ca / exchangeable Mg[(d)]		7.34	High. Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		0.58	High Unfavorable for plant nutrition in Mg
Extractable iron (Fe)[(f)]	mg kg^{-1}	58.51	High
Extractable copper (Cu)[(f)]	mg kg^{-1}	1.31	Medium
Extractable zinc (Zn)[(f)]	mg kg^{-1}	4.29	High
Extractable manganese (Mn)[(f)]	mg kg^{-1}	14.06	Low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Table 5. The classification of the rootzone mixture with cork "earth" (CE) according to the organic matter (OM) content, the pH and electrical conductivity (EC) value, the extractable phosphorus (P_2O_5) and potassium (K_2O), the exchangeable bases (Ca, Mg, Na, K), the cation exchange capacity (CEC), relation between exchangeable Ca:Mg, and exchangeable K:Mg, and the extractable micronutrients (Fe, Cu, Zn, Mn) contents.

Parameters	Units of measure	Cork "earth" (CE)	Observations
Organic matter (OM)[(a)]	%	2.01	Medium
pH (H_2O) (1:2.5)[(b)]		6.42	Little acidic
Electrical conductivity (EC) (1:2)[(c)]	mS cm^{-1}	0.13	Soil without saline effects
Extractable phosphorus (P_2O_5)[(b)]	mg kg^{-1}	46.19	Low (Fertility index 2)
Extractable potassium (K_2O)[(b)]	mg kg^{-1}	222.57	Very high (Fertility index 7)
Calcium (exchangeable base) (Ca)[(a)]	cmol(+) kg^{-1}	1.99	Low
Magnesium (extractable base) (Mg)[(a)]	cmol(+) kg^{-1}	0.27	Very low
Sodium (extractable base) (Na) [(a)]	cmol(+) kg^{-1}	0.01	Very low
Potassium (extractable base) (K)[(a)]	cmol(+) kg^{-1}	0.47	Medium
Cationic Exchange Capacity (CEC)[(a)]	cmol(+) kg^{-1}	2.82	Very low
Relation exchangeable Ca / exchangeable Mg[(d)]		7.37	High. Unfavorable for plant nutrition in Mg
Relation exchangeable K / exchangeable Mg		1.74	High Unfavorable for plant nutrition in Mg
Extractable iron (Fe)[(f)]	mg kg^{-1}	22.59	Low
Extractable copper (Cu)[(f)]	mg kg^{-1}	0.35	Low
Extractable zinc (Zn)[(f)]	mg kg^{-1}	1.29	Low
Extractable manganese (Mn)[(f)]	mg kg^{-1}	12.01	Low

(a) Classification according to Alves (1989).
(b) Classification according to Soveral-Dias *et al*. (1980).
(c) Classification adapted from Isaac *et al*. (1983).
(d) Classification adapted from Ilaco (1981).
(e) Classification adapted from Roselem *et al*. (1984).
(f) Classification adapted from Sillanpää (1982); Costa & Fernandes (1996) and Costa *et al*. (1999).

Annex III

The development of *Lolium perenne* cv. ‘Benchmark’ and *Agrostis stolonifera* cv. ‘Penn-A4’ and the soil surface coverage, in the beginning and at the end of the experiment, in the four rootzone mixtures: peat (PT), sewage sludge with pine bark compost (NA), green compost (NV) and cork “earth” (CE).

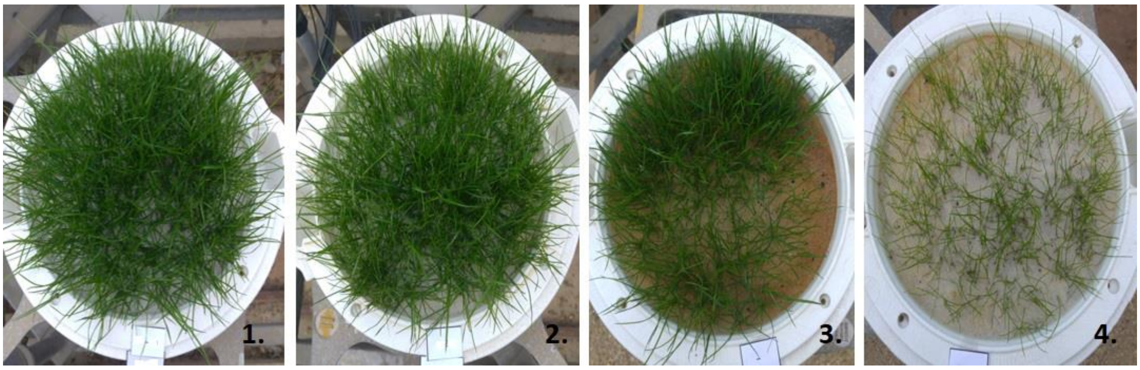

Figure 1.The development *Lolium perenne* cv. ‘Benchmark’ before the first harvest (June 7^{th}) and the soil surface coverage, in the respective rootzone mixture: 1) peat (PT); 2) sewage sludge with pine bark compost (NA); 3) green compost (NV and 4) cork “earth” (CE).

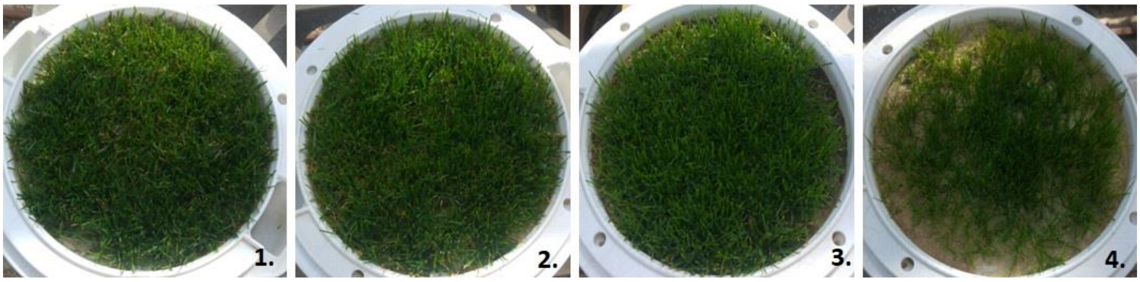

Figure 2. The development of *Lolium perenne* cv. ‘Benchmark’ at the end of the experiment (July 19th) and the soil surface coverage, in the respective rootzone mixture: 1) peat (PT); 2) sewage sludge with pine bark compost (NA); 3) green compost (NV and 4) cork “earth” (CE).

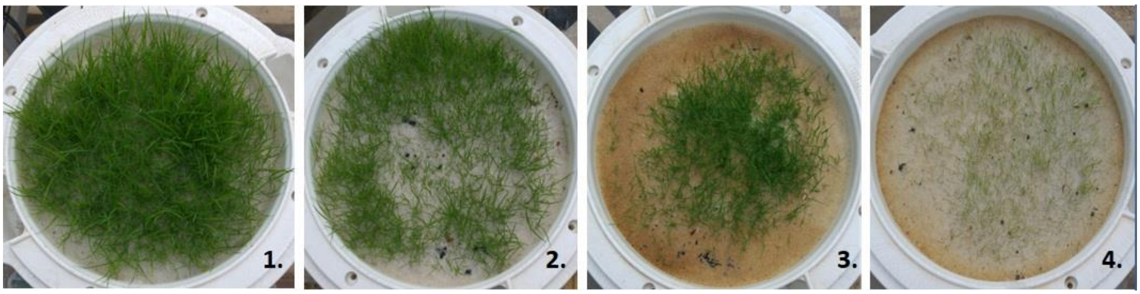

Figure 3. The development of *Agrostis stolonifera* cv. ‘Penn-A4’ a week before its first shoot harvest (June 7th) and the soil surface coverage, in the respective rootzone mixture: 1) peat (PT); 2) sewage sludge with pine bark compost (NA); 3) green compost (NV and 4) cork “earth” (CE).

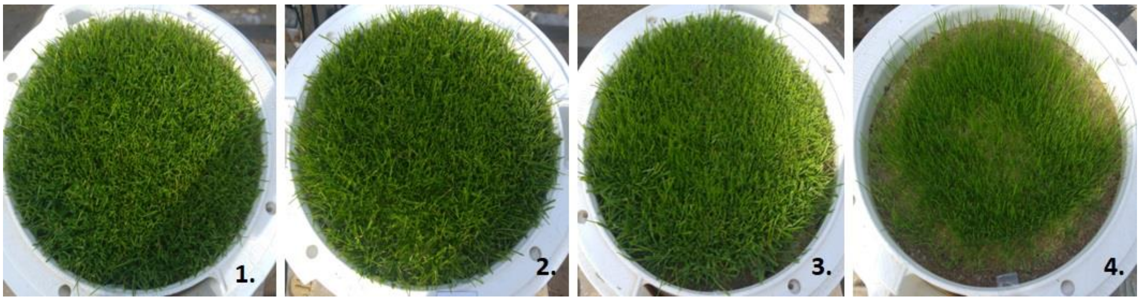

Figure 4. The development of *Agrostis stolonifera* cv. 'Penn-A4' at the end of the experiment (July 24th) and the soil coverage, in the respective rootzone mixture: 1) peat (PT); 2) sewage sludge with pine bark compost (NA); 3) green compost (NV and 4) cork "earth" (CE).

Annex IV

The identified weeds present throughout the experiment, in the respective rootzone mixtures: peat (PT), sewage sludge with pine bark compost (NA) and cork “earth” (CE).

Figure 1. *Senecio vulgaris* (left) and *Amaranthus albus* (right), growing in the rootzone mixture with cork "earth" (CE).

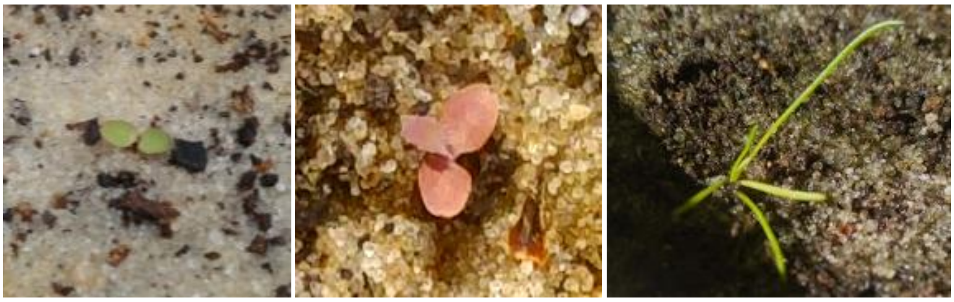

Figure 2. *Solanum nigrum*, *Sonchus oleraceus* and *Spergula arvensis* growing in the control pot with the rootzone mixture cork “earth” (CE).

Figure 3. *Ageratina riparia* growing in the control pot with the rootzone mixture with peat (PT).

Figure 4. *Sonchus oleraceus* growing in the control pot with the rootzone mixture with sewage sludge and pine bark compost (NA).